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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 31/715, C07G 17/00, C07H 1/00</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 96/40169</b> <b>(43) International Publication Date:</b> 19 December 1996 (19.12.96)
<b>(21) International Application Number:</b> PCT/US96/08333 <b>(22) International Filing Date:</b> 3 June 1996 (03.06.96) <b>(30) Priority Data:</b> 488,053 7 June 1995 (07.06.95) US <b>(71) Applicant:</b> NEOSE TECHNOLOGIES, INC. [US/US]; 102 Witmer Road, Horsham, PA 19044 (US). <b>(72) Inventors:</b> SIMON, Paul, M.; 307 Irving Drive, Wilmington, DE 19802 (US). ZOPF, David; 560 W. Beechtree Lane, Strafford, PA 19087 (US). BARTHELSON, Roger, A.; 377 Park Drive West, Kintnersville, PA 18930 (US). JOHNSON, Karl, F.; 2941 Windsor Avenue, Willow Grove, PA 19090 (US). <b>(74) Agents:</b> CORUZZI, Laura, A. et al.; Pennie & Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US).		<b>(81) Designated States:</b> AL, AM, AU, AZ, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IL, IS, JP, KG, KP, KR, KZ, LK, LR, LS, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> BACTERIAL INHIBITION WITH AN OLIGOSACCHARIDE COMPOUND		
<div style="text-align: center;"> <p style="text-align: right;">(I)</p> </div> <div style="text-align: center;"> <p style="text-align: right;">(II)</p> </div>		
<b>(57) Abstract</b> <p>The present invention is directed to a method of treating respiratory infections caused by a bacteria selected from the group consisting of <i>S. pneumoniae</i>, <i>H. influenzae</i>, <i>H. parainfluenzae</i>, <i>Burkholderia</i> (<i>Pseudomonas cepacia</i>) and a mixture thereof, by administering a binding inhibiting effective amount of the compound of formula (I), where R<sub>1</sub> is H, (β-1)GalNAc-, SO<sub>3</sub>B (where B is H or a cation) or a sialic acid of formula (II), where R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub>, and R<sub>10</sub> are each independently H, C<sub>1-6</sub> acyl, lactyl, C<sub>1-6</sub> alkyl, sulfate, phosphate, anhydro, a sialic acid of formula (II), (α-1)Fuc, (β-1)Glc or (β-1)Gal; R<sub>9</sub> is NH-C<sub>1-6</sub> acyl, glycolylamido, amino or hydroxyl; and A is H or a cation; R<sub>2</sub> is H or (α-1)Fuc-; R<sub>3</sub> and R<sub>4</sub> are each independently OH or NHAc; R<sub>5</sub> is H, SO<sub>3</sub>B (where B is H or a cation) or a sialic acid of formula (II) as defined above; and Y is a chemical bond or a linking group; Z is H or a multivalent support; m is 0 or 1; and p is an integer of 1 to 1,000; and a pharmaceutical composition containing same.</p>		

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TITLE OF THE INVENTION

BACTERIAL INHIBITION WITH AN OLIGOSACCHARIDE COMPOUND

BACKGROUND OF THE INVENTION5 Field of the Invention:

The present invention is directed to a method of treating bacterial infections caused by a bacteria selected from the group consisting of *Streptococcus pneumoniae*, *Haemophilus influenza*, *Hemophilus parainfluenza*, *Burkholderia*  
10 (*Pseudomonas*) *cepacia* and a mixture thereof using an oligosaccharide, as well as a pharmaceutical composition containing same.

Discussion of the Background:

*S. pneumoniae*, *H. influenza*, *H. parainfluenza*, and  
15 *Burkholderia* (*Pseudomonas*) *cepacia* are pathogenic bacteria associated with respiratory diseases such as pneumonia, bacterial bronchitis, conjunctivitis, sinusitis and otitis media. In each of these conditions, the bacteria colonizes the healthy tissue of the nasopharynx, conjunctiva, lung,  
20 bronchi or ear canal, establishing a large enough population to cause the disease. The bacteria adhere to the cell-surfaces, at specific receptor sites for the bacteria.

Conventional therapy has relied on administration of antibiotics, in order to alleviate the bacterial colonization.  
25 However, antibiotic therapy is subject to the development of antibiotic resistant strains, (and can ultimately generate "super bacteria") which would not be treatable with known antibiotics. A report issued by the American Society of

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Microbiology, in May of 1995, indicated that more and more infectious organisms are becoming untreatable with antibiotics and that steps must be taken to reverse this trend before a public health crisis occurs.

5           Researchers have studied the interactions of pathogenic bacteria with tissue receptors, responsible for adherence, and have identified specific carbohydrate sequences which can inhibit bacterial adherence.

Krivan et al U.S. 5,386,027, report binding inhibition of  
10       specific "opportunistic pathogenic bacteria", which are associated with respiratory tract infections, with compounds containing either an internal or terminal GalNAc $\beta$ 1-4-Gal $\beta$ 1-4-Glc sequence. The specific pulmonary pathogenic bacteria for which binding inhibition are reported are *Pseudomonas*  
15       *aeruginosa*, *Haemophilus influenza*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Escherichia coli*. The reference postulates that ganglioside GM1 is present in substantial amounts in human lung tissue and that pulmonary pathogenic bacteria require at least a terminal  
20       or internal GalNAc $\beta$ 1-4-Gal, which is unsubstituted with sialyl residues.

Andersson et al J. Exp. Med. (1983), v. 158, p. 559-570, report binding inhibition studies on *Streptococcus pneumoniae*, and identify a disaccharide GlcNAc  $\beta$ 1-3-Gal $\beta$  as a specific  
25       glycoconjugate receptor. The reference reports that compound 3, NeuAc $\alpha$ 2-3Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc-Cer had no effect at binding inhibition (page 564, first sentence under Table IV).

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Rosenstein et al, Infection and Immunity (1992), vol. 60, no. 12, 5078-5084, report binding specificities for *Pseudomonas aeruginosa* M35 and *Escherichia coli* C600, isolated from cystic fibrosis patients for various oligosaccharide structures. Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc was reported to have high binding activity while NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc showed no activity.

Ramphal et al, Infection and Immunity (1991) vol. 59, no. 2, p. 700-704, report binding inhibition studies for *Pseudomonas aeruginosa* to Type 1 (Gal $\beta$ 1-3-GlcNAc) and Type 2 (Gal $\beta$ 1-4GlcNAc) disaccharides. NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc is reported to have no activity in the direct adhesion assay.

Ginsburg et al U.S. 5,225,330 report a diagnostic device for absorbing microorganisms, using specific carbohydrate receptor sequences. The reference reports no activity for sialylparagoboside in binding inhibition of *Mycoplasma pneumoniae* (Table I, col. 11) but reports *Escherichia coli* inhibition of N-glycolylsialoparagloboside.

Feizi et al Biochemistry, 1994, 33, 6342-6349, report that NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc is not bound or is only negligibly bound by immunoglobulin E-binding protein, while NeuAc $\alpha$ 2-3-Gal $\beta$ 1-3-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc is strongly bound by this protein.

Magnani et al WO92/18610 report the use of sialyl-Le<sup>a</sup> (Neu5Ac $\alpha$ 2-3-Gal $\beta$ 1-3[Fuc $\alpha$ 1-4]GlcNAc) and sialyl-Le<sup>x</sup> (Neu5Ac $\alpha$ 2-3-Gal $\beta$ 1-4[Fuc $\alpha$ 1-3]GlcNAc) in inhibiting binding of malignant cells to endothelial cells both in vivo and in vitro.

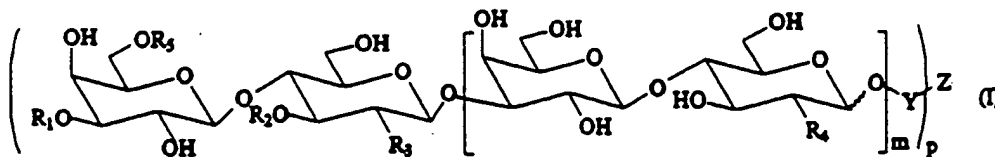
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Andersson et al J. of Infectious Diseases (1986), vol. 153, no. 2, 232-237, report that fractions of human milk inhibited the attachment of *Streptococcus pneumoniae* and *Haemophilus influenzae* to human pharyngeal or buccal epithelial cells.

Accordingly, new methods of treating bacterial infections of a bacteria selected from the group consisting of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Burkholderia (Pseudomonas) cepacia* and a mixture thereof, are being sought, which have a lower probability of generating antibiotic resistant strains. Anti-adhesion compounds are potentially useful candidates in this search.

#### SUMMARY OF THE INVENTION

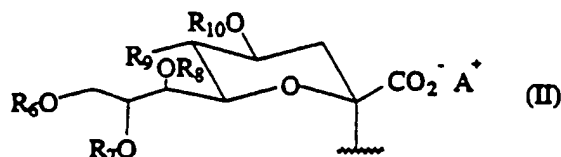
Accordingly, one object of this invention is to provide a novel method of inhibiting colonization of a bacteria selected from the group consisting of *S. pneumoniae*, *H. influenzae*, *H. parainfluenzae*, *Burkholderia (Pseudomonas) cepacia* and a mixture thereof, by administering a binding inhibiting effective amount of the compound of Formula I,



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where

$R_1$  is H, ( $\beta$ -1)GalNAc-,  $\text{SO}_3\text{B}$  (where B is H or a cation) or a sialic acid of Formula II;



where

$R_6$ ,  $R_7$ ,  $R_8$ , and  $R_{10}$  are each independently H,  $\text{C}_{1-6}$  acyl, lactyl,  $\text{C}_{1-6}$  alkyl, sulfate, phosphate, anhydro, a sialic acid of Formula II, ( $\alpha$ -1)Fuc, ( $\beta$ -1)Glc or ( $\beta$ -1)Gal;

$R_9$  is  $\text{NH-C}_{1-6}$  acyl, glycolylamido, amino or hydroxyl; and

A is H or a cation;

$R_2$  is H or ( $\alpha$ -1)Fuc-;

$R_3$  and  $R_4$  are each independently OH or NHAc;

$R_5$  is H,  $\text{SO}_3\text{B}$  (where B is H or a cation) or a sialic acid of Formula II as defined above; and

Y is a chemical bond or a linking group;

Z is H or a multivalent support;

m is 0 or 1; and

p is an integer of 1 to 1,000.

A second object of this invention is to provide a method of inhibiting bacterial colonization of a bacteria selected from the group consisting of *S. pneumoniae*, *H. influenza*, *H.*

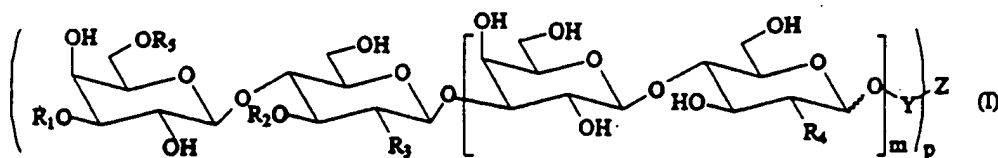
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*parainfluenzae*, *Burkholderia* (*Pseudomonas*) *cepacia* and a mixture thereof in a patient diagnosed with pneumonia.

Another object of this invention is to provide a method of inhibiting colonization of a bacteria selected from the group consisting of *S. pneumoniae*, *H. influenza*, *H. parainfluenzae*, *Burkholderia (Pseudomonas) cepacia* and a mixture thereof in a patient diagnosed with bacterial bronchitis.

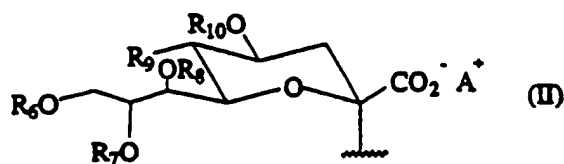
Another object of the invention is to provide a method of  
10 inhibiting bacterial colonization of a bacteria selected from  
the group consisting of *S. pneumoniae*, *H. influenza*, *H.*  
*parainfluenzae*, *Burkholderia* (*Pseudomonas*) *cepacia* and a  
mixture thereof in a patient diagnosed with otitis media.

Another object of this invention is to provide a novel  
15 pharmaceutical composition containing a compound of Formula I.



where

R<sub>1</sub> is H, (β-1)GalNac-, SO<sub>3</sub>B (where B is H or a cation) or a sialic acid of Formula II;





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where

$R_6$ ,  $R_7$ ,  $R_8$ , and  $R_{10}$  are each independently H,  $C_{1-6}$  acyl, lactyl,  $C_{1-6}$  alkyl, sulfate, phosphate, anhydro, a sialic acid of Formula II, ( $\alpha$ -1)Fuc, ( $\beta$ -1)Glc or ( $\beta$ -1)Gal;

$R_9$  is  $NH-C_{1-6}$  acyl, glycolylamido, amino or hydroxyl; and

A is H or a cation;

$R_2$  is H or ( $\alpha$ -1)Fuc-;

$R_3$  and  $R_4$  are each independently OH or NHAc;

$R_5$  is H,  $SO_3B$  (where B is H or a cation) or a sialic acid of Formula II as defined above; and

Y is a chemical bond or a linking group;

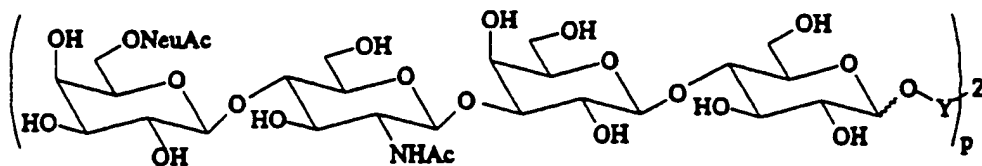
Z is H or a multivalent support;

m is 0 or 1; and

p is an integer of 1 to 1,000

wherein when  $R_5$  is H,  $R_1$  is not H and when  $R_1$  is a sialic acid,  $R_2$  is not ( $\alpha$ -)Fuc.

In a preferred embodiment, the compound of Formula I is of the formula



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where

Y is a chemical bond or a linking group;

$Z$  is  $H$  or a multivalent support; and

p is an integer of 1 to 1,000.

5           These and other objects of the present invention are enabled by the inventors' discovery that a bacteria selected from the group consisting of *S. pneumoniae*, *H. influenza*, *H. parainfluenzae*, *Burkholderia* (*Pseudomonas*) *cepacia* and a mixture thereof can be inhibited from colonizing, by a  
10       compound of Formula I.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following abbreviations are used throughout the text:

"Gal" for galactose; "Glc" to glucose, "GlcNac" for N-acetylglucosamine; "GalNac" for N-acetylgalactosamine; "Fuc" for fucose; "NAN or NeuAc" for N-acetylneuraminic acid.

Specific oligosaccharide compounds may include NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-

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4-GlcNAc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc and GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc.

A preferred group of oligosaccharide compounds are the above-identified group, excluding the compound Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc.

Each of these compounds also can be administered in monovalent (i.e.  $p = 1$ ) or in multivalent form (i.e.  $p = 2-1,000$ ), either with or without a linker group Y. In addition, the linking group Y can be bonded directly to Z where Z is H, or a multivalent support.

The oligosaccharides of the present invention may be obtained using any known method, including (1) enzymatically, using one of the method described in U.S. 5,180,674, (2) synthetically, using classical organic chemistry, (3) by degradation of a natural occurring oligosaccharide, glycolipid, or glycopeptide or (4) isolation from natural source such as human milk. The isolation of LSTc is reported by Kuhn, R. and Gauche, A. Chem Ber., 95, 513 (1962) and Dorland et al. Eur. J. Bioch. 87, 323 (1978)

For example the compound NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, can be prepared enzymatically from lactose. Accordingly lactose is reacted with a  $\beta$  galactoside  $\beta$ 1-3 N-acetylglucosaminyltransferase and UDP-GlcNAc, a  $\beta$  N-acetylglucosaminoside  $\beta$ 1-4 galactosyltransferase and UDP-Gal and a  $\beta$  galactoside  $\alpha$ 2-3 sialyltransferase and CMP-NeuAc under

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suitable conditions to affect transfer of the three sugar groups from the sugar nucleotides to the appropriate acceptor molecule.

The groups  $R_1$  and  $R_2$  can be a group  $SO_3B$ , where B can be either H or a cation. Accordingly, the compound of Formula I includes sulfate substitute and salts thereof. Suitable cations include alkali metals, alkaline earth metals or ammonium. Any known suitable pharmaceutically acceptable cations may be used, including the cations of conventional non-toxic salts including a metal salt such as an alkali metal salt (e.g. sodium salt, potassium salt, etc.) or an alkaline earth metal salt (e.g. calcium salt, magnesium salt, etc.), an ammonium salt, an organic base salt (e.g. trimethylamine salt, triethylamine salt, pyridine salt, picoline salt, dicyclohexylamine salt, N, N'-dibenzylethylenediamine salt, etc.), an organic acid salt (e.g. formate, acetate, trifluoroacetate, maleate, tartrate, methanesulfonate, benzenesulfonate, toluenesulfonate, etc.), an inorganic acid salt (e.g. hydrochloride, hydrobromide, sulfate, phosphate, etc.), a salt with an amino acid (e.g. arginine salt, aspartic acid salt, glutamic acid salt, etc.), and the like.

The compound of Formula I can also be the corresponding aza sugar, in which the oxygen of one or more of the pyran rings is replaced with a nitrogen, to form a piperidine ring system.

The aza sugars corresponding to Formula I can be prepared by conventional methods known to those of ordinary skill in the art such as through classical organic chemistry techniques

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or enzymatically using the appropriate aza saccharide as the acceptor substrate. Aza sugar nucleotides can be transferred by the corresponding glycosyltransferase for the natural sugar. Aza glucose can be isolated from natural sources and  
5 can be converted to the aza lactose by the action of a galactosyltransferase in the presence of a galactose donor such as UDP-gal.

The compound of Formula I can also be the corresponding thio sugar, in which the oxygen of one or more of the pyran  
10 rings is replaced with a sulfur, to form a tetrahydrothiopyran ring system.

The thio sugars corresponding to Formula I can be prepared by conventional methods known to those of ordinary skill in the art such as through classical organic chemistry  
15 techniques or enzymatically using the appropriate thio saccharide as the acceptor substrate. A monothiosaccharide can be prepared by conventional classical organic chemical techniques from the corresponding monosaccharide.

The compound of Formula II is a sialic acid, which is a  
20 family of 9-carbon carboxylated sugars related to neuraminic acid. The carboxylic acid may be in the form of a free acid, when A is H or a salt, when A is a cation.

Suitable cations include alkali metals, alkaline earth metals or ammonium. Any known suitable pharmaceutically  
25 acceptable cations may be used, including the cations of conventional non-toxic salts including a metal salt such as an alkali metal salt (e.g. sodium salt, potassium salt, etc.) or an alkaline earth metal salt (e.g. calcium salt, magnesium

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salt, etc.), an ammonium salt, an organic base salt (e.g. trimethylamine salt, triethylamine salt, pyridine salt, picoline salt, dicyclohexylamine salt, N, N'-dibenzylethylenediamine salt, etc.), an organic acid salt  
5 (e.g. formate, acetate, trifluoroacetate, maleate, tartrate, methanesulfonate, benzenesulfonate, toluenesulfonate, etc.), an inorganic acid salt (e.g. hydrochloride, hydrobromide, sulfate, phosphate, etc.), a salt with an amino acid (e.g. arginine salt, aspartic acid salt, glutamic acid salt, etc.),  
10 and the like.

Preferably the compound of Formula II is selected from the group consisting of N-acetyl-neuraminic acid, N-glycolyl-neuraminic acid, keto-deoxy-nonulosonic acid, 9-O-acetyl N-acetyl-neuraminic acid, 9-O-acetyl N-glycolyl-neuraminic acid,  
15 9-O-acetyl-keto-deoxy-nonulosonic, 7-O-acetyl-N-acetyl-neuraminic acid, 7-O-acetyl-N-glycolyl-neuraminic acid, 4-O-acetyl-N-acetyl-neuraminic acid, 4-O-acetyl-N-glycolyl-neuraminic acid, 7,9-di-O-acetyl-N-acetyl-neuraminic acid, 8,9-di-O-acetyl-N-acetyl-neuraminic acid, 7,9-di-O-acetyl-N-glycolyl-neuraminic acid, 8,9-di-O-acetyl-N-glycolyl-neuraminic acid, 4,9-di-O-acetyl-N-acetyl-neuraminic acid,  
20 7,8,9-tri-O-acetyl-N-acetyl-neuraminic acid, 7,8,9-tri-O-acetyl-N-glycolyl-neuraminic acid, 9-O-lactyl-N-acetyl-neuraminic acid, 9-O-lactyl-N-glycolyl-neuraminic acid, 4-O-acetyl-9-O-lactyl-N-acetyl-neuraminic acid, 4-O-acetyl-9-O-lactyl N-glycolyl-neuraminic acid, 8-O-methyl-N-acetyl-neuraminic acid, 8-O-methyl-N-glycolyl-neuraminic acid, 8-O-methyl-9-O-acetyl-N-glycolyl-neuraminic acid, 8-O-methyl-7,9-

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di-O-acetyl-N-glycolyl-neuraminic acid, 8-O-sulpho-N-glycolyl-neuraminic acid, 8-O-phosphoro-N-acetyl-neuraminic acid, 2,3 didehydro 2,6 anhydro-N-acetyl-neuraminic acid, 9-O-acetyl-2,3 didehydro 2,6 anhydro-N-acetyl-neuraminic acid, 9-O-lactyl-2,3 didehydro 2,6 anhydro-N-acetyl-neuraminic acid, 2,3 didehydro 2,6 anhydro-N-glycolyl-neuraminic acid, 9-O-acetyl-2,3 didehydro 2,6 anhydro-N-glycolyl-neuraminic acid, 9-O-lactyl-2,3 didehydro 2,6 anhydro-N-glycolyl-neuraminic acid, 8-O-methyl-2,3 didehydro 2,6 anhydro-N-glycolyl-neuraminic acid, 2,7 anhydro-N-acetyl-neuraminic acid, 2,7 anhydro-N-glycolyl-neuraminic acid, 8-O-methyl-2,7 anhydro-N-glycolyl-neuraminic acid, 4,8 anhydro-N-acetyl-neuraminic acid and salts thereof. More preferably the sialic acid of Formula II is N-acetyl-neuraminic acid or N-glycolyl-neuraminic acid. These sialic acids are described in A.Varki Glycobiology v2, (1992) p25-40. Accordingly the reference describes sources of the sialic acids as well as the appropriate sialyltransferase necessary for enzymatic synthesis of oligosaccharides of Formula I.

When the compound of Formula II is substituted with a sialic acid, the substitution is preferably at the R<sub>7</sub> position.

The linking group Y is any group which serves to link the oligosaccharide portion of the compound of Formula I, with the group Z. Suitable linking groups include saccharides, oligosaccharides, peptides, proteins, C<sub>2-20</sub> alkyl, oxyalkylene chains or any other group, which does not diminish the binding inhibiting activity of the oligosaccharide portion of the compound of Formula I. In addition, the linking group can be

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a chemical bond, in which case the oligosaccharide portion of the compound of Formula I is directly bound to the group Z.

The oligosaccharide can be provided as a multivalent molecule according to Formula I (i.e.  $p = 2-1,000$ ). In this embodiment the oligosaccharide portion is bound to a multivalent support using known techniques so as to produce a conjugate in which more than one individual molecule of the oligosaccharide is covalently attached, through the group Y to the multivalent support. The multivalent support is sufficiently long to provide a multivalent molecule leaving from between 2-1,000 (i.e.  $p = \text{an integer of } 2-1,000$ ), preferably 2-100, more preferably 2-50 molecules of the oligosaccharide portion bound to the multivalent support.

A suitable multivalent support is a compound with multiple binding sites, to a terminal end of the linking group which is not bound to the reducing end saccharide, or with multiple binding sites to the C<sub>1</sub> glycosidic oxygen of a glucose or N-acetylglucosamine residue. Examples include but are not limited to a polyol, a polysaccharide, polylysine, avidin, a polyacrylamide, dextran, lipids, lipid emulsions, liposomes, a dendrimer, human serum albumin, bovine serum albumin or a cyclodextrin.

The chemistry necessary to link the reducing end saccharide (i.e., Glc or GlcNAc) with the linking group Y and to link the linking group Y to the multivalent support is well known in the field of linking chemistry. For example a bond between the reducing end saccharide and Y can be formed by reacting an aldehyde or carboxylic acid at C<sub>1</sub> of the reducing



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end saccharide or any aldehyde or carboxylic acid group introduced onto the reducing end saccharide by oxidation, with the Y group, to form a suitable bond such as -NH-, -N(R')- where R' is C<sub>1-20</sub> alkyl, a hydroxyalkylamine, a amide, an ester, a thioester, a thioamide.

The bond between the reducing end saccharide and Y can be formed by reacting the C<sub>1</sub> hydroxyl group, in the pyranose form, with an acylating agent and a molecular halide, followed by reaction with a nucleophile to form a suitable bond such as -NH-, -N(R')- where R' is C<sub>1-20</sub> alkyl, -S- and -O-. This type of linking chemistry is described by Stowell et al Advances in Carbohydrate Chemistry and Biochemistry, 37 (1980) p 225+.

The oligosaccharide portion can be bound directly (i.e Y is a chemical bond) to the multivalent support via the free anomeric carbon of the reducing end saccharide.

Alternatively, the reducing end saccharide can be bound via a phenethylamine-isothiocyanate derivative as described by Smith et al, Complex Carbohydrates part C, Methods in Enzymology, volume L, Ed by V. Ginsburg (1978), p 169-171. It is preferable that the oligosaccharide of Formula I remain soluble in water. It is also possible however to administer the oligosaccharide of Formula I in the form of a suspension in a suitable carrier.

The method of inhibiting bacterial colonization is achieved by administering a binding inhibiting effective amount of the oligosaccharide of Formula I, to a patient in need thereof. The method of administration will be dependant on the desired site of delivery of the lig saccharide. For

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example to treat respiratory infections, the oligosaccharide is administered to the nasopharynx, lung or bronchi, preferably in the form of an aerosol. To treat otitis media, the oligosaccharide is administered to the nasopharynx, preferably in the form of drops. To treat conjunctivitis, the oligosaccharide can be administered to the conjunctive tissue, preferably in the form as eye drops or an eye ointment.

Suitable compositions may take the form of a solution, suspension or any pharmaceutically acceptable form suitable for delivery to the site of infection, especially the lungs, bronchial passage, nasopharynx or middle ear. The oligosaccharide can also be administered to the lungs and bronchial passage in the form of an aerosolized dry powder.

The pharmaceutical compositions are usually administered as a mixture with a carrier suitably selected depending upon the route for administration using standard formulations.

A solution or suspension may be prepared by adding any diluent customarily, used in the art. For example, suitable diluents include water (bacteriostatic, sterile and/or pyrogen free), ethyl alcohol, propylene glycol, polyoxyethylene sorbitol, and sorbitan esters. Sodium chloride, glucose or glycerol may be incorporated into such a liquid preparation in an amount sufficient to prepare an isotonic solution.

The oligosaccharides of Formula I may also be administered in conjunction with an antibiotic with activity against a bacteria selected from the group consisting of *S. pneumoniae*, *H. influenza*, *H. parainfluenzae*, *Burkholderia* (*Ps udomonas*) *cepacia* and a mixture thereof. Examples of

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suitable classes of antibiotics include aminoglycosides, amphenicols, ansamycins,  $\beta$ -lactams such as carbapenems, cephalosporins, cephamycins, monobactams, oxacephems and penicillins, lincosamides, macrolides, polypeptides, tetracyclines, 2,4-diaminopyrimidines, nitrofurans, quinolones, sulfonamides, sulfones,  $\beta$ -lactamase inhibitors and antifungal antibiotics such as polyenes. Specific examples of suitable antibiotic compounds include, but are not limited to metronidazole, tetracycline, bismuth, erythromycin, sulfonamide, penicillin, cephalosporin, amoxicilin, cycloserine, fosfomycin, vancomycin, bacitracin, polymyxins, mitomycin, novobiocin, actinomycin, rifampicin, clindamycin, lincomycin, isoniazid, flucytosine, chloramphenicol, puromycin, fusidic acid, griseofulvin, p-aminosalicylic acid, trimethoprim, imipenem. Suitable antibiotics also include antibiotic compounds as identified in the Merck Index, Eleventh edition, especially those listed on pages THER-9, THER-10 and THER-13, the entire contents of which are hereby incorporated by reference. The amount of antibiotic administered in conjunction with the present oligosaccharide is about the same amount administered for its known therapy. Accordingly, effective dosage of the antibiotic can be determined by routine experimentation.

The therapeutic composition may also further contain ordinary dissolving aids, buffers, pain-alleviating agents, art preservatives, and optionally coloring agents, fragrances, flavors, sweeteners and other pharmacologically active agents such as are well known in the art.

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The pharmaceutical compositions are preferably formulated to provide an isotonic solution. The pH of such compositions is preferably at a pH of from 6 to 8, preferably from 6.8 to 7.7, more preferably at physiological pH.

5           Typically, suitable patients are humans. However the present method is also applicable to treatment of animals, including but not limited to mammals such as pigs, cows, horses, sheep, goats, dogs, cats, rodents and non-human primates.

10           According to a preferred embodiment of the present invention, the oligosaccharide containing pharmaceutical composition is administered as an aerosol to a patient in need thereof to inhibit binding or eliminate colonies of a bacteria selected from the group consisting of *S. pneumoniae*, *H.*  
15   *influenza*, *H. parainfluenzae*, *Burkholderia* (*Pseudomonas*) *cepacia* and a mixture thereof from the patient's throat, conjunctiva, lungs or bronchial passage.

For aerosol administration, the oligosaccharide compound of Formula I, is preferably supplied in finely divided form  
20   along with a surfactant and propellant. Typical amounts of the oligosaccharide of Formula I are about 0.5 to 30 wt.%, preferably about 1 to 10 wt.%. The surfactant is nontoxic and preferably is soluble in the propellant. Representative of  
25   such surfactant agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, lesteric, and oleic acids, with an aliphatic polyhydric alcohol or its cyclic anhydride such as, for

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example, ethylene glycol, glycerol, erythritol, arabitol, mannitol, sorbitol, the hexitol anhydrides derived from sorbitol, and the polyoxyethylene and polyoxypropylene derivatives of these esters. Mixed esters, such as mixed or  
5 natural glycerides can be employed. The surfactant can constitute about 0.1 to about 20 percent by weight of the composition, and preferably about 0.25 to about 5 percent.

The balance of the composition is ordinarily propellant. Liquified propellants are typically gases at ambient  
10 conditions, and are condensed under pressure. Among suitable liquified propellants are the lower alkanes containing up to 5 carbons, such as butane and propane; and preferably fluorinated or a fluorochlorinated alkane such as R-11. Mixtures of the above can also be employed. In producing the  
15 aerosol, a container equipped with a suitable valve is filled with the appropriate propellant, containing the finely divided compounds and surfactant. The ingredients are thus maintained at an elevated pressure until released by action of the valve.

For administration as an aerosol, generation of such an  
20 aerosol is accomplished by conventional means, known to those of ordinary skill in the art of drug delivery.

When administered as an aerosol in monovalent form, for the treatment of a respiratory infection, the composition is formulated to provide a concentration in the mucus membranes  
25 of from 0.0001 to 20 mg/mL, preferably 0.01 to 10 mg/mL.

When administered as a multivalent molecule for the treatment of a respiratory infection, an aerosol pharmaceutical composition comprising the oligosaccharide of

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Formula I is administered so as to achieve a concentration in the mucus membranes of 0.00001 to 2 mg/mL, preferably 0.001 to 1 mg/mL.

According to another embodiment of the present invention,  
5 the oligosaccharide containing pharmaceutical composition is administered via the nasopharynx to the ear of a patient in need thereof to inhibit binding, or eliminate colonies of bacteria selected from the group consisting of *S. pneumoniae*,  
10 *H. influenza*, *H. parainfluenzae*, *Burkholderia* (*Pseudomonas*) *cepacia* and a mixture thereof from a patient's middle ear. A patient in need thereof is typically diagnosed with otitis media.

The mode of administration is such as to deliver a binding inhibiting effective amount of the oligosaccharide of  
15 Formula I, to the site of infection. This can typically be achieved by administering nasal drops or spray containing the oligosaccharide of Formula I, via the nasopharynx, to the ear. The oligosaccharide of Formula I can also be administered in the form of an oral mouthwash, in which the oligosaccharide is  
20 delivered to the site of infection by the action of gargle and reflux.

When administered to the ear, a suitable form is a liquid solution or suspension in a non-toxic pharmaceutically acceptable diluent or solvent, for example, as a solution in  
25 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils are conventionally employed as a solvent or suspending medium.

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For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. In addition, fatty acids such as oleic may find use in the preparation of injectables.

5           When administered to the ear in monovalent form, for the treatment of otitis media, the composition is formulated to provide a concentration at the site of infection of from 0.0001 to 20 mg/mL, preferably 0.01 to 10 mg/mL.

          When administered as a multivalent molecule for the  
10       treatment of otitis media, the composition is formulated to provide a concentration at the site of infection of 0.00001 to 2 mg/mL, preferably 0.001 to 1 mg/mL.

          According to another embodiment of the present invention, the oligosaccharide containing pharmaceutical composition is  
15       administered to the eye to a patient in need thereof to inhibit binding or eliminate colonies of a bacteria selected from the group consisting of *S. pneumoniae*, *H. influenza*, *H. parainfluenzae*, *Burkholderia* (*Pseudomonas*) *cepacia* and a mixture thereof from a patient's eye. A patient in need  
20       thereof is typically diagnosed with conjunctivitis.

          When administered to the eye, a suitable form is a liquid solution or suspension in a non-toxic pharmaceutically acceptable diluent or solvent, for example, as an ophthalmic saline solution.

25           When administered to the eye in monovalent form, for the treatment of conjunctivitis, the composition is formulated to provide a concentration at the site of infection of from 0.0001 to 20 mg/mL, preferably 0.01 to 10 mg/mL.

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When administered as a multivalent molecule for the treatment of conjunctivitis, the composition is formulated to provide a concentration at the site of infection of 0.00001 to 2 mg/mL, preferably 0.001 to 1 mg/mL.

5        These dosages can be achieved by administration, at least daily, preferably twice daily, more preferably three times a day and most preferably four times a day.

      The present invention can also be used to prevent a bacterial infection of *S. pneumoniae*, *H. influenza*, *H.*  
10    *parainfluenzae* and *Burkholderia (Pseudomonas) cepacia*, in a patient in need thereof. While it is generally desirable to prevent a bacterial infection in all patients who are asymptomatic for a bacterial infection, there is an identifiable population, for which the consequences of a  
15    bacterial infection make the risk of a bacterial infection unacceptable. Specifically, the consequences of a bacterial infection in the young, old or immunocompromised are especially devastating. Accordingly, the oligosaccharide of Formula I can be administered to a patient in need of  
20    prevention of a bacterial infection of *S. pneumoniae*, *H. influenza*, *H. parainfluenzae* and *Burkholderia (Pseudomonas) cepacia* in an amount sufficient to prevent bacterial infection.

      For the prevention of a respiratory bacterial infection,  
25    the oligosaccharide of Formula I is typically administered in monovalent form to provide a concentration in the mucus membranes of from 0.0001 to 20 mg/mL, preferably 0.01 to 10



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mg/mL. These prophylactic dosages can be achieved by administration, at least daily, preferably twice daily.

When administered as a multivalent molecule for the prevention of a respiratory infection, an aerosol pharmaceutical composition comprising the oligosaccharide of Formula I is administered so as to achieve a concentration in the mucus membranes of 0.00001 to 2 mg/mL, preferably 0.001 to 1 mg/mL.

When administered via the nasopharynx to the ear for the prevention of otitis media, the composition is formulated in a monovalent form to provide a concentration in the membranes of the ear of from 0.0001 to 20 mg/mL, preferably 0.01 to 10 mg/mL.

When administered via the nasopharynx as a multivalent molecule to the ear for the prevention of otitis media, for treating otitis media comprising the oligosaccharide of Formula I is administered so as to achieve a concentration at the membranes of the ear of 0.00001 to 2 mg/mL, preferably 0.001 to 1 mg/mL.

The composition of the pharmaceutical composition of the invention, is basically as described above for the inhibition of bacterial infections. The amount of the oligosaccharide of Formula I will vary depending on the mode of administration, based on the amount necessary to deliver a binding inhibiting effective amount to the site of the infection. Generally the pharmaceutical will contain the oligosaccharide at a concentration of about 0.01 to 2,000 mg/mL, preferably 1 to

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1,000 mg/mL in monovalent form, and from 0.001 to 200 mg/mL, preferably from 0.1 to 100 mg/mL in multivalent form.

Other features of the invention will become apparent in the course of the following descriptions of exemplary  
5   embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

Throughout the examples section the following abbreviations are used:

LSTc: NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc  
10   LNnT: Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc  
LSTd (3'LSTc): NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc  
GalNAc  $\beta$ 1-3 LNnT: GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc  
HSA: human serum albumin

Example 1

15   Synthesis of NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc

Lactose was contacted with UDP-N-acetylglucosamine and a  $\beta$ -galactoside  $\beta$ 1-3 N-acetylglucosaminyl transferase purified from pig serum in a 0.1 M HEPS buffered aqueous solution at 37°C. The product trisaccharide was then contacted with UDP-  
20   Gal and a  $\beta$ -N-acetylglucosaminoside  $\beta$ 1-4 Galactosyltransferase isolated from bovine milk, in a 0.1 M HEPS buffered aqueous solution at 37°C. The product tetrasaccharide was then contacted with CMP-NAN and a  $\beta$ -galactoside  $\alpha$ 2-6 sialyltransferase isolated from rat liver in a 0.1 M HEPES  
25   buffered aqueous solution at 37°C. The title pentasaccharide was isolated by conventional methods.

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Example 2

A method similar to that of Rostand, K. and Esko, (1993) J. Biol. Chem. 268 pp 24053-3, which studied the binding of radiolabelled *P. aeruginosa* to CHO cells was used to test for the binding inhibition of oligosaccharide compounds. For the studies described herein, *S. pneumoniae* (strains R-6, P70, and other clinical respiratory isolates) were labelled by culture in lysine-deficient tryptic soy broth containing  $^3\text{H}$ -lysine. The tryptic soy broth was made deficient in lysine by pre-treating it with lysine decarboxylase from *E.coli*. The bacteria were cultured for 4-5 hours, at which point the culture was in exponential growth. The bacteria were harvested and washed in Leibovitz L-15 Medium supplemented with 0.2% low-endotoxin bovine serum albumin. The bacteria were diluted to approximately  $3 \times 10^8/\text{mL}$ , and then mixed 1:1 with either L-15-BSA or with various compounds to be tested. The bacterial mixtures were incubated at room temperature, with gentle agitation on an orbital shaker. After 10-15 min, samples of the bacterial mixtures were transferred to the surface of a confluent monolayer of cultured epithelial cells in a 96-well polystyrene plate. The cells most commonly used were the Wong-Kilbourne derivative of Chang conjunctiva. The human nasopharyngeal cell line Detroit 562, and the human lung cell line A549 were also used. The bacteria were incubated with the cells for 30 min with gentle agitation, at room temperature. The unbound bacteria were washed away with PBS containing 0.2% bovine serum albumin. Scintillant solution

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was added to the wells, and the plate was put in a microplate scintillation counter for quantitation of bound bacteria.

Adhesion of *H. influenzae* to epithelial cells was performed with the same protocol with the following  
 5 modifications: The bacteria were cultured overnight in lysine-deficient *Haemophilus* test media. The bacteria were incubated with the cells at a concentration near  $10^7$ /mL for 60 min.

IC<sub>50</sub> (mM)10 LSTc

Bacteria	Det-562	FaDu	CaLu-3	1° lung	A549	HUVEC	CHANG
<i>S. pneumoniae</i>	5.4	4.0	4.4	0.4	0.1	0.1	1.5
<i>P. cepacia</i>			1				

15 LSTc-HSA<sup>3</sup>

Bacteria	Det-562	FaDu	CaLu-3	1° lung	A549	HUVEC
<i>S. pneumoniae</i>	0.10		0.0004			
<i>H. influenzae</i>	0.03					

20

Det-562 and FaDu: nasopharyngeal carcinomas

CaLu-3 and A459<sup>1</sup>: lung carcinomas

1°lung: rat tracheal primary epithelium

HUVEC<sup>2</sup>: human umbilical vein endothelial cells

25 <sup>1</sup> cells activated with IL1 $\alpha$

<sup>2</sup> cells activated with TNF $\alpha$  or thrombin

<sup>3</sup> LSTc bound to a human serum albumin backbone; the molar ratio of oligosaccharide:prot = 20:1. Oligosaccharide molarity is given.

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Inhibition of adherence of *S. pneumoniae* to respiratory epithelial cells

IC<sub>50</sub> (mM)  
[mean (SD)]

5 Monovalent

	CELL LINE <sup>1</sup>						
	Det-562	FaDu	CaLu-3	1°lung	A549 <sup>2</sup>	HUVEC <sup>3</sup>	Chang
LNnT	7.0		>16.0		0.095 (0.005)	0.052 (0.022)	8.0
LSTc	4.0	4.5	4.4	0.25	0.069 (0.032)	0.069 (0.035)	1.4
LSTd (3°LSTc)					0.095 (0.005)	0.090	0.6
10 GalNAc β1-3 LNnT					0.080	0.075	
GalNAc β1-3 Galβ1-4 Glc					0.085	0.070	>6.0

Multivalent<sup>4</sup>

	CELL LINE <sup>1</sup>					
	Det-562	FaDu	CaLu-3	1°lung	A549 <sup>2</sup>	HUVEC <sup>3</sup>
15 LNnT	>0.500				0.100	0.030 (0.020)
LSTc	0.125	0.160	0.110	0.250	0.021 (0.006)	0.023 (0.012)
GalNAc β1-3 Gal-HSA	0.500	0.020	0.100	0.0004	0.100	0.087 (0.018)

- 20 1. Detroit-562 and FaDu: human nasopharyngeal carcinoma  
CaLu-3 and A549: human lung carcinoma  
HUVEC: human umbilical vein endothelial cells (primary)  
Rat trachea epithelial monolayer (primary)  
Chang: human conjunctival carcinoma
- 25 2. Activated with IL1α  
3. Activated with TNF<sub>α</sub> or thrombin  
4. Human serum albumin backbone; molar ratio of oligosaccharide:prot = 20:1. Oligosaccharide molar concentrations are given.

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Example 3In vivo experiments with LSTc and LNnT.

Rabbits. *S. pneumoniae* strain AII were mixed with oligosaccharides and administered to New Zealand white rabbits by tracheal instillation, and lung lavage colonies were counted at the indicated time by the method described in Saukkonen, et al., 1991, J. Exp. Med. 173:1143-9.

Colonization of rabbit lungs with *S. pneumoniae* in the presence of an adherence-inhibitory oligosaccharide

	Conditions	CFU*		
		LSTc (100 $\mu$ M)	LNnT (100 $\mu$ M)	Control (saline)
10	1 Inoculum: $5 \times 10^5$ bacteria; intratracheal; 24 h lavage	$1.4 \times 10^5$ , $9.1 \times 10^3$	$8.7 \times 10^2$ , $4.7 \times 10^3$	$7.5 \times 10^4$ , $7.1 \times 10^3$
	4 day lavage	<10, <10	<10, <10	$1.6 \times 10^4$ , $4.5 \times 10^3$
	2 Inoculum: $5 \times 10^5$ bacteria intratracheal 4 day lavage	0, 0	0, 0	$1.6 \times 10^4$ , $4.5 \times 10^3$
	3 Inoculum: $5 \times 10^7$ bacteria**; intratracheal; 24 h lavage	0, 0	0, 0	$3.1 \times 10^5$ , $2.2 \times 10^4$
	4 Inoculum: $10^5$ bacteria; intratracheal; 24 h lavage		$1.1 \times 10^3$ , $1.4 \times 10^3$	$1.4 \times 10^5$ , $1.4 \times 10^5$

\* Lungs were lavaged on day 4 and fluid was quantitatively plated on blood agar for counting of bacterial colonies/ml lavage fluid. 2 rabbits/group.

\*\* Saline control animals given this high inoculum became very sick, with lungs heavily colonized. One rabbit was bacteremic ( $10^4$  CFU/ml blood). Olig saccharide treated animals were healthy throughout the experiment.

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Example 4Treatment of rabbits pre-colonized with *S. pneumoniae* with adherence-reversing oligosaccharide.

*S. pneumoniae* strain AII were administered to New Zealand white rabbits by tracheal instillation. All rabbits were sick at 24h, with indications of pneumonia. After 24h oligosaccharide (LNnT) or control (saline) was administered intratracheally. After an additional 24h lung lavage colonies were counted as described in Example 3.

10	Conditions	CFU	
		LNnT (100 $\mu$ M)	Control (Saline)
15	Inoculum: $10^5$ bacteria intratracheal lavage 24h after oligosaccharide treatment and 48h after bacterial instillation	0,0	$5.4 \times 10^3$ , $4.8 \times 10^3$

At 48h the LNnT-treated rabbits were healthy, whereas saline control rabbits were ill, with bloody, congested lungs.

Example 5

Rats: Newborn Sprague-Dawley rats were inoculated with  $10^6$  *S. pneumoniae* strain SIII intranasally in a volume of 20  $\mu$ l saline. At the indicated time nasal cavity was lavaged and plated over blood agar in serial dilutions for determination of the number of colonies.

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Colonization of rat nasopharynx with *S. pneumoniae* in the presence of an adherence- inhibitory oligosaccharide

		CFU*		
	Conditions	LSTc-HSA (100 $\mu$ M)	LNnT-HSA (100 $\mu$ M)	Control (saline)
1	Inoculum: $10^6$ bacteria; intranasal; 4 rats/group; 3 hour nasal lavage	$4.1(\pm 1.0) \times 10^4$	$3.5(\pm 0.4) \times 10^4$	$8.8(\pm 0.8) \times 10^4$
	7 day nasal lavage	$3 \pm 1$	$6 \pm 1$	$268 \pm 62$
2	Inoculum: $10^6$ bacteria; intranasal; 8 rats/group 5.5 h lavage	$4.1(\pm 0.8) \times 10^4$	$3.7(\pm 0.9) \times 10^4$	$8.3(\pm 0.8) \times 10^4$

- 5        \*        Nasal cavity was lavaged at the indicated times and the number of CFU/ml lavage fluid was determined.

Example 6

Using the testing protocol described in Example 2, four strains of bacteria were tested for binding inhibition by

10        different oligosaccharide compounds.



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Inhibition of adherence of other pathogenic bacteria to respiratory epithelial cells

$$\frac{IC_{50} (mM)}{[mean (SD)]}$$

5	Bacteria	H. influenzae (strain 46519)	H. influenzae (strain 39689)	H. parainfluenzae (strain 44241)	Burkholderi a (Pseudomona s)cepacia
	CELLS	Chang	Chang	DET-562	Calu 3
	LNnT	> 14.0	> 14.0		5.6
	LSTc		> 10.0		1
10	GalNAc $\beta$ 1-3 LNnT	7.8 (2.9)	> 10.0		
	GalNAc $\beta$ 1-3 Gal $\beta$ 1-4 Glc	4.2 (5.3)	10.6 (7.7)		
	LNnT-HSA	> 0.2	> 0.2	0.10	
	LSTc-HSA		> 0.2	0.03	
15	GalNAc $\beta$ 1-3 Gal-BSA			0.25	

\* Highest concentration tested is 10-15 mM monovalent oligosaccharides and 0.2-0.25 mM multivalent oligosaccharides, for which the carbohydrate molar concentration is given.

20 Example 7

10 mL of an isotonic pharmaceutical composition comprising 500 mg of NeuAca2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, 100 mL of sterile water, and 10 mL of propylene glycol is administered to a human patient diagnosed with pneumonia, in the form of an aerosol. Treatment is continued three times a day, at eight hour intervals, until the bacterial infection has been eliminated.

Example 8

5 dr ps of a pharmaceutical comp sition comprising 1,000 mg of NeuAca2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc and 100 mL of 1,3-

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butanediol is administered to the nasopharynx of a human patient diagnosed with otitis media, in the form of drops or nasal spray. Treatment is continued twice a day, at twelve hour intervals, until the bacterial infection has been  
5 eliminated.

#### Example 9

5 drops of an ophthalmic solution comprising 800 mg of NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc and 100 mL of isotonic saline solution is administered to the eye of a human  
10 patient diagnosed with conjunctivitis, in the form of eye drops. Treatment is continued four times a day, at six hour intervals, until the bacterial infection has been eliminated.

#### Example 10

10 mL of an isotonic pharmaceutical composition comprising  
15 500 mg of GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, 100 mL of sterile water, and 10 mL of propylene glycol is administered to a human patient diagnosed with pneumonia, in the form of an aerosol. Treatment is continued three times a day, at eight hour intervals, until the bacterial infection has been  
20 eliminated.

#### Example 11

10 mL of an isotonic pharmaceutical composition comprising  
500 mg of NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, 100 mL of sterile water, and 10 mL of propylene glycol is administered  
25 to a human patient diagnosed with pneumonia, in the form of an

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aerosol. Treatment is continued three times a day, at eight hour intervals, until the bacterial infection has been eliminated.

Example 12

- 5        10 mL of an isotonic pharmaceutical composition comprising  
200 mg of NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc-HSA, at an  
oligosaccharide:prot ratio of 20:1, 100 mL of sterile water,  
and 10 mL of propylene glycol is administered to a human  
patient diagnosed with pneumonia, in the form of an aerosol.  
10    Treatment is continued twice daily, at twelve hour intervals,  
until the bacterial infection has been eliminated.

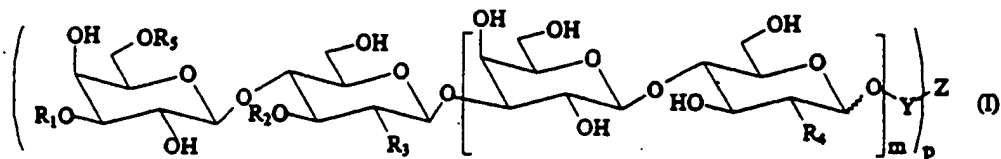
\* \* \* \* \*

- Obviously, numerous modifications and variations of the  
present invention are possible in light of the above  
15    teachings. It is therefore to be understood that within the  
scope of the appended claims, the invention may be practiced  
otherwise than as specifically described herein.

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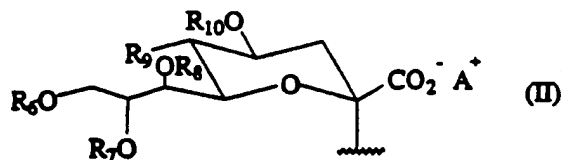
WHAT IS CLAIMED AS NEW AND DESIRED TO BE SECURED BY LETTERS  
PATENT OF THE UNITED STATES IS:

1. A method of inhibiting colonization of a bacteria selected from the group consisting of *S. pneumoniae*, *H. influenza*, *H. parainfluenzae*, *Burkholderia* (*Pseudomonas*) *cepacia* and a mixture thereof, by administering a binding inhibiting effective amount of the compound of Formula I,



where

- 10  $R_1$  is H, ( $\beta$ -1)GalNAc-,  $\text{SO}_3\text{B}$  (where B is H or a cation) or a sialic acid of Formula II;



where

- 15  $R_6$ ,  $R_7$ ,  $R_8$ , and  $R_{10}$  are each independently H,  $\text{C}_{1-6}$  acyl, lactyl,  $\text{C}_{1-6}$  alkyl, sulfate, phosphate, anhydro, a sialic acid of Formula II, ( $\alpha$ -1)Fuc, ( $\beta$ -1)Glc or ( $\beta$ -1)Gal;  
 $R_9$  is  $\text{NH-C}_{1-6}$  acyl, glycolylamido, amino or hydroxyl;  
 and

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A is H or a cation;

$R_2$  is H or  $(\alpha-1)\text{Fuc-}$ ;

$R_3$  and  $R_4$  are each independently OH or NHAc;

$R_5$  is H,  $\text{SO}_3\text{B}$  (where B is H or a cation) or a sialic acid

5 of Formula II as defined above; and

Y is a chemical bond or a linking group;

Z is H or a multivalent support;

m is 0 or 1; and

p is an integer of 1 to 1,000.

10 2. The method Claim 1, wherein said patient is infected with bacteria selected from the group consisting of *S. pneumoniae*, *H. influenza*, *H. parainfluenzae*, *Burkholderia (Pseudomonas) cepacia*, and a mixture thereof.

15 3. The method Claim 2, wherein said patient has bronchitis.

4. The method Claim 2, wherein said patient has pneumonia.

5. The method Claim 1, wherein said patient has otitis media.

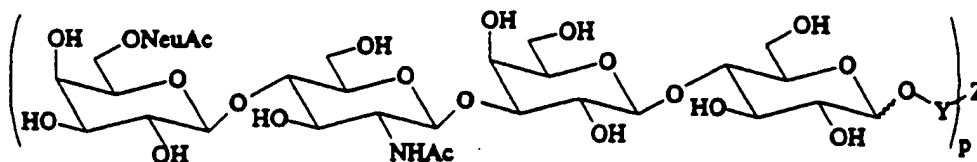
20 6. The method of Claim 1, wherein said compound of Formula I is selected from the group consisting of NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-

25

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3]GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, and a mixture thereof.

7. The method of Claim 1, wherein said compound of Formula I is of the formula



where

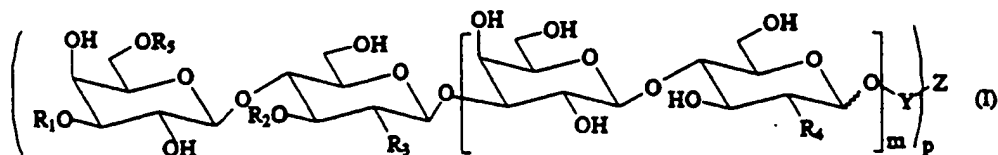
Y is a chemical bond or a linking group;  
Z is H or a multivalent support; and  
p is an integer of 1 to 1,000.

8. The method of Claim 7, wherein Y is a chemical bond, Z is H and p is 1.

9. A pharmaceutical composition for inhibiting colonization of bacteria selected from the group consisting of *S. pneumoniae*, *H. influenzae*, *H. parainfluenzae*, *Burkholderia (Pseudomonas) cepacia*, and a mixture thereof, by administering

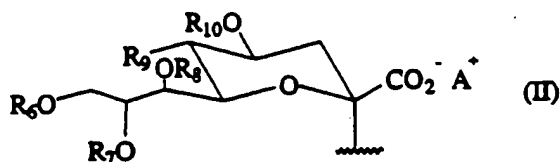
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a binding inhibiting effective amount of the compound of  
Formula I,



where

- 5  $R_1$  is H, ( $\beta$ -1)GalNAC-,  $SO_3B$  (where B is H or a cation) or  
a sialic acid of Formula II;



where

- 10  $R_6$ ,  $R_7$ ,  $R_8$ , and  $R_{10}$  are each independently H,  $C_{1-6}$   
acyl, lactyl,  $C_{1-6}$  alkyl, sulfate, phosphate,  
anhydro, a sialic acid of Formula II, ( $\alpha$ -1)Fuc, ( $\beta$ -  
1)Glc or ( $\beta$ -1)Gal;  
 $R_9$  is  $NH-C_{1-6}$  acyl, glycolylamido, amino or hydroxyl;  
and  
15 A is H or a cation;  
 $R_2$  is H or ( $\alpha$ -1)Fuc-;  
 $R_3$  and  $R_4$  are each independently OH or NHAc;  
 $R_5$  is H,  $SO_3B$  (where B is H or a cation) or a sialic acid  
of Formula II as defined above; and

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Y is a chemical bond or a linking group;

Z is H or a multivalent support;

m is 0 or 1; and

p is an integer of 1 to 1,000

5        wherein when  $R_2$  is H,  $R_1$  is not H and when  $R_1$  is a sialic acid,  $R_2$  is not ( $\alpha$ -)Fuc.

10        10. The composition of Claim 9, wherein said compound of Formula I is selected from the group consisting of NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, and a mixture thereof.

25        11. The pharmaceutical composition of Claim 9, wherein said composition is in a form suitable for administration as an aer sol.



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/08333

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : A61K 31/715; C07G 17/00; C07H 1/00

US CL : 514/53, 54; 536/123, 123.1, 123.13

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/53, 54; 536/123, 123.1, 123.13

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CARBBANK, HCAPLUS

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	WO 95/23605 A1 (NEOSE PHARMACEUTICALS, INC.) 08 September 1995, see entire document.	9-11
Y	KITAGAWA et al. Cloning of a Novel alpha-2,3-Sialyltransferase That Sialylates Glycoprotein and Glycolipid Carbohydrate Groups. The Journal of Biological Chemistry. 14 January 1994, Vol. 269, No. 2, pages 1394-1401, see especially page 1400.	9-11
Y	AJISAKA et al. Regioselective transglycosylation in the synthesis of oligosaccharides: comparison of beta-galactosidases and sialidases of various origins. Carbohydrate Research. 1994, Vol. 259, pages 103-115, see entire document.	9-11

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

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*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
13 AUGUST 1996	27 AUG 1996
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Facsimile No. (703) 305-3230	Telephone No. (703) 308-1235

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/08333

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	RAMPHAL et al. <i>Pseudomonas aeruginosa</i> Recognizes Carbohydrate Chains Containing Type 1 (Gal-beta1-3GlcNAc) or Type 2 (Gal-beta1-4GlcNAc) Dissaccharide Units. <i>Infection and Immunity</i> . February 1991, Vol. 59, No. 2, pages 700-704, see entire document.	9-11 1-8
X Y	ANDERSSON et al. Inhibition of Attachment of <i>Streptococcus pneumoniae</i> and <i>Haemophilus influenzae</i> by Human Milk and Receptor Oligosaccharides. <i>The Journal of Infectious Diseases</i> . February 1986, Vol. 153, No. 2, pages 232-237, see entire document.	9-11 1-8
Y	GRONBERG et al. Isolation of Monosialylated Oligosaccharides From Human Milk and Structural Analysis of Three New Compounds. <i>Carbohydrate Research</i> . 1989, Vol. 191, pages 261-278, see especially pages 264 and 266.	9-11
Y	PAULSEN et al. Synthese Der Tetra- Und Trisaccharid-Sequenzen Von Asialo-GM1 Und GM2 Lenkung Der Regioselektivitat Der Glycosidierung Von Lactose. <i>Carbohydrate Research</i> . 1985, Vol. 137, pages 39-62, see especially pages 44 and 47.	9-11
Y	LIUKKONEN et al. Identification of N-Acetylneuraminy1 alpha2->3 Poly-N-acetyllactosamine Glycans as the Receptors of Sialic Acid-binding <i>Streptococcus suis</i> Strains. <i>The Journal of Biological Chemistry</i> . 15 October 1992, Vol. 267, No. 29, pages 21105-21111, see especially page 21105.	9-11
Y	FEIZI et al. Further definition of the size of the blood group-i antigenic determinant using a chemically synthesised octasaccharide of poly-N-acetyllactosamine type. <i>Carbohydrate Research</i> . 1992, Vol. 228, pages 289-297, see especially page 289.	9-11



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification <sup>6</sup> : <b>A61K 31/715, C07G 17/00, C07H 1/00</b></p>	<p><b>A1</b></p>	<p>(11) International Publication Number: <b>WO 96/40169</b></p> <p>(43) International Publication Date: 19 December 1996 (19.12.96)</p>
<p>(21) International Application Number: <b>PCT/US96/08333</b></p> <p>(22) International Filing Date: <b>3 June 1996 (03.06.96)</b></p> <p>(30) Priority Data: <b>488,053</b>                      <b>7 June 1995 (07.06.95)</b>                      <b>US</b></p> <p>(71) Applicant: <b>NEOSE TECHNOLOGIES, INC. [US/US]; 102 Witmer Road, Horsham, PA 19044 (US).</b></p> <p>(72) Inventors: <b>SIMON, Paul, M.; 307 Irving Drive, Wilmington, DE 19802 (US). ZOPF, David; 560 W. Beechtree Lane, Strafford, PA 19087 (US). BARTHELSON, Roger, A.; 377 Park Drive West, Kintnersville, PA 18930 (US). JOHNSON, Karl, F.; 2941 Windsor Avenue, Willow Grove, PA 19090 (US).</b></p> <p>(74) Agents: <b>CORUZZI, Laura, A. et al.; Pennie &amp; Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US).</b></p>		<p>(81) Designated States: <b>AL, AM, AU, AZ, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IL, IS, JP, KG, KP, KR, KZ, LK, LR, LS, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</b></p> <p><b>Published</b> <i>With international search report.</i></p>
<p>(54) Title: <b>BACTERIAL INHIBITION WITH AN OLIGOSACCHARIDE COMPOUND</b></p>		
<div style="text-align: center;"> <p style="text-align: right;">(I)</p> </div> <div style="text-align: center; margin-top: 20px;"> <p style="text-align: right;">(II)</p> </div>		
<p>(57) Abstract</p> <p>The present invention is directed to a method of treating respiratory infections caused by a bacteria selected from the group consisting of <i>S. pneumoniae</i>, <i>H. influenzae</i>, <i>H. parainfluenzae</i>, <i>Burkholderia</i> (<i>Pseudomonas cepacia</i>) and a mixture thereof, by administering a binding inhibiting effective amount of the compound of formula (I), where R<sub>1</sub> is H, (β-1)GalNAc-, SO<sub>3</sub>B (where B is H or a cation) or a sialic acid of formula (II), where R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub>, and R<sub>10</sub> are each independently H, C<sub>1-6</sub> acyl, lactyl, C<sub>1-6</sub> alkyl, sulfate, phosphate, anhydro, a sialic acid of formula (II), (α-1)Fuc, (β-1)Glc or (β-1)Gal; R<sub>9</sub> is NH-C<sub>1-6</sub> acyl, glycolylamido, amino or hydroxyl; and A is H or a cation; R<sub>2</sub> is H or (α-1)Fuc-; R<sub>3</sub> and R<sub>4</sub> are each independently OH or NHAc; R<sub>5</sub> is H, SO<sub>3</sub>B (where B is H or a cation) or a sialic acid of formula (II) as defined above; and Y is a chemical bond or a linking group; Z is H or a multivalent support; m is 0 or 1; and p is an integer of 1 to 1,000; and a pharmaceutical composition containing same.</p>		

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GA	Gabon			VN	Viet Nam

TITLE OF THE INVENTION

BACTERIAL INHIBITION WITH AN OLIGOSACCHARIDE COMPOUND

BACKGROUND OF THE INVENTION5 Field of the Invention:

The present invention is directed to a method of treating bacterial infections caused by a bacteria selected from the group consisting of *Streptococcus pneumoniae*, *Haemophilus influenza*, *Haemophilus parainfluenza*, *Burkholderia*  
10 (*Pseudomonas*) *cepacia* and a mixture thereof using an oligosaccharide, as well as a pharmaceutical composition containing same.

Discussion of the Background:

*S. pneumoniae*, *H. influenza*, *H. parainfluenza*, and  
15 *Burkholderia* (*Pseudomonas*) *cepacia* are pathogenic bacteria associated with respiratory diseases such as pneumonia, bacterial bronchitis, conjunctivitis, sinusitis and otitis media. In each of these conditions, the bacteria colonizes the healthy tissue of the nasopharynx, conjunctiva, lung,  
20 bronchi or ear canal, establishing a large enough population to cause the disease. The bacteria adhere to the cell-surfaces, at specific receptor sites for the bacteria.

Conventional therapy has relied on administration of antibiotics, in order to alleviate the bacterial colonization.  
25 However, antibiotic therapy is subject to the development of antibiotic resistant strains, (and can ultimately generate "super bacteria") which would not be treatable with known antibiotics. A report issued by the American Society of

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Microbiology, in May of 1995, indicated that more and more infectious organisms are becoming untreatable with antibiotics and that steps must be taken to reverse this trend before a public health crisis occurs.

5           Researchers have studied the interactions of pathogenic bacteria with tissue receptors, responsible for adherence, and have identified specific carbohydrate sequences which can inhibit bacterial adherence.

Krivan et al U.S. 5,386,027, report binding inhibition of  
10       specific "opportunistic pathogenic bacteria", which are associated with respiratory tract infections, with compounds containing either an internal or terminal GalNAc $\beta$ 1-4-Gal $\beta$ 1-4-Glc sequence. The specific pulmonary pathogenic bacteria for which binding inhibition are reported are *Pseudomonas*  
15       *aeruginosa*, *Haemophilus influenza*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Escherichia coli*. The reference postulates that ganglioside GM1 is present in substantial amounts in human lung tissue and that pulmonary pathogenic bacteria require at least a terminal  
20       or internal GalNAc $\beta$ 1-4-Gal, which is unsubstituted with sialyl residues.

Andersson et al J. Exp. Med. (1983), v. 158, p. 559-570, report binding inhibition studies on *Streptococcus pneumoniae*, and identify a disaccharide GlcNAc  $\beta$ 1-3-Gal $\beta$  as a specific  
25       glycoconjugate receptor. The reference reports that compound 3, NeuAc $\alpha$ 2-3Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc-Cer had no effect at binding inhibition (pag 564, first sentence under Table IV).

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Rosenstein et al, Infection and Immunity (1992), vol. 60, no. 12, 5078-5084, report binding specificities for *Pseudomonas aeruginosa* M35 and *Escherichia coli* C600, isolated from cystic fibrosis patients for various oligosaccharide structures. Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc was reported to have high binding activity while NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc showed no activity.

Ramphal et al, Infection and Immunity (1991) vol. 59, no. 2, p. 700-704, report binding inhibition studies for *Pseudomonas aeruginosa* to Type 1 (Gal $\beta$ 1-3-GlcNAc) and Type 2 (Gal $\beta$ 1-4GlcNAc) disaccharides. NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc is reported to have no activity in the direct adhesion assay.

Ginsburg et al U.S. 5,225,330 report a diagnostic device for absorbing microorganisms, using specific carbohydrate receptor sequences. The reference reports no activity for sialylparagoboside in binding inhibition of *Mycoplasma pneumoniae* (Table I, col. 11) but reports *Escherichia coli* inhibition of N-glycolylsialoparagloboside.

Feizi et al Biochemistry, 1994, 33, 6342-6349, report that NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc is not bound or is only negligibly bound by immunoglobulin E-binding protein, while NeuAc $\alpha$ 2-3-Gal $\beta$ 1-3-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc is strongly bound by this protein.

Magnani et al WO92/18610 report the use of sialyl-Le<sup>a</sup> (Neu5Ac $\alpha$ 2-3-Gal $\beta$ 1-3[Fuc $\alpha$ 1-4]GlcNAc) and sialyl-Le<sup>x</sup> (Neu5Ac $\alpha$ 2-3-Gal $\beta$ 1-4[Fuc $\alpha$ 1-3]GlcNAc) in inhibiting binding of malignant cells to endothelial cells both in vivo and in vitro.

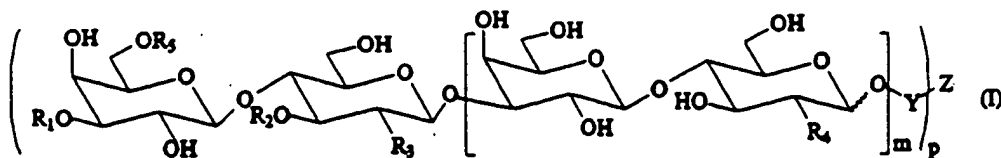
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Andersson et al J. of Infectious Diseases (1986), vol. 153, no. 2, 232-237, report that fractions of human milk inhibited the attachment of *Streptococcus pneumoniae* and *Haemophilus influenzae* to human pharyngeal or buccal epithelial cells.

Accordingly, new methods of treating bacterial infections of a bacteria selected from the group consisting of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Burkholderia (Pseudomonas) cepacia* and a mixture thereof, are being sought, which have a lower probability of generating antibiotic resistant strains. Anti-adhesion compounds are potentially useful candidates in this search.

#### SUMMARY OF THE INVENTION

Accordingly, one object of this invention is to provide a novel method of inhibiting colonization of a bacteria selected from the group consisting of *S. pneumoniae*, *H. influenzae*, *H. parainfluenzae*, *Burkholderia (Pseudomonas) cepacia* and a mixture thereof, by administering a binding inhibiting effective amount of the compound of Formula I,

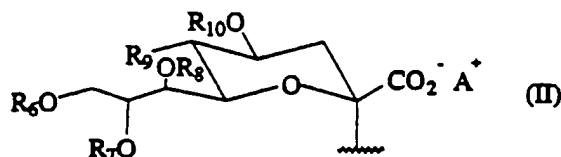




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where

$R_1$  is H, ( $\beta$ -1)GalNAc-,  $\text{SO}_3\text{B}$  (where B is H or a cation) or a sialic acid of Formula II;



where

$R_6$ ,  $R_7$ ,  $R_8$ , and  $R_{10}$  are each independently H,  $\text{C}_{1-6}$  acyl, lactyl,  $\text{C}_{1-6}$  alkyl, sulfate, phosphate, anhydro, a sialic acid of Formula II, ( $\alpha$ -1)Fuc, ( $\beta$ -1)Glc or ( $\beta$ -1)Gal;

$R_9$  is  $\text{NH-C}_{1-6}$  acyl, glycolylamido, amino or hydroxyl; and

A is H or a cation;

$R_2$  is H or ( $\alpha$ -1)Fuc-;

$R_3$  and  $R_4$  are each independently OH or NHAc;

$R_5$  is H,  $\text{SO}_3\text{B}$  (where B is H or a cation) or a sialic acid of Formula II as defined above; and

Y is a chemical bond or a linking group;

Z is H or a multivalent support;

m is 0 or 1; and

p is an integer of 1 to 1,000.

A second object of this invention is to provide a method of inhibiting bacterial colonization of a bacteria selected from the group consisting of *S. pneumoniae*, *H. influenza*, *H.*

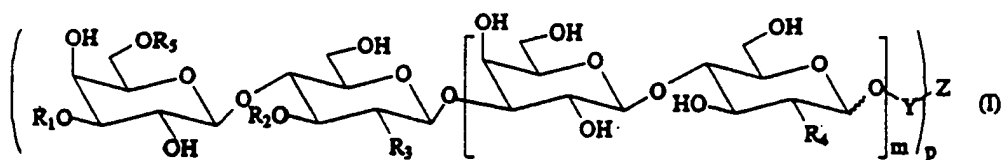
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*parainfluenzae*, *Burkholderia* (*Pseudomonas*) *cepacia* and a mixture thereof in a patient diagnosed with pneumonia.

Another object of this invention is to provide a method of inhibiting colonization of a bacteria selected from the group consisting of *S. pneumoniae*, *H. influenza*, *H. parainfluenzae*, *Burkholderia (Pseudomonas) cepacia* and a mixture thereof in a patient diagnosed with bacterial bronchitis.

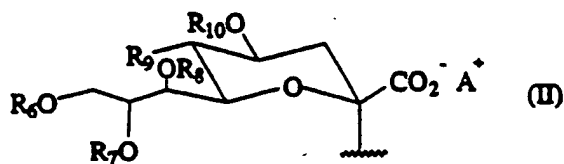
Another object of the invention is to provide a method of  
10 inhibiting bacterial colonization of a bacteria selected from  
the group consisting of *S. pneumoniae*, *H. influenza*, *H.*  
*parainfluenzae*, *Burkholderia* (*Pseudomonas*) *cepacia* and a  
mixture thereof in a patient diagnosed with otitis media.

Another object of this invention is to provide a novel  
15 pharmaceutical composition containing a compound of Formula I.



**where**

R<sub>1</sub> is H, (β-1)GalNAc-, SO<sub>3</sub>B (where B is H or a cation) or a sialic acid of Formula II;



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where

$R_6$ ,  $R_7$ ,  $R_8$ , and  $R_{10}$  are each independently H,  $C_{1-6}$  acyl, lactyl,  $C_{1-6}$  alkyl, sulfate, phosphate, anhydro, a sialic acid of Formula II, ( $\alpha$ -1)Fuc, ( $\beta$ -1)Glc or ( $\beta$ -1)Gal;

$R_9$  is  $NH-C_{1-6}$  acyl, glycolylamido, amino or hydroxyl; and

A is H or a cation;

$R_2$  is H or ( $\alpha$ -1)Fuc-;

$R_3$  and  $R_4$  are each independently OH or NHAc;

$R_5$  is H,  $SO_3B$  (where B is H or a cation) or a sialic acid of Formula II as defined above; and

Y is a chemical bond or a linking group;

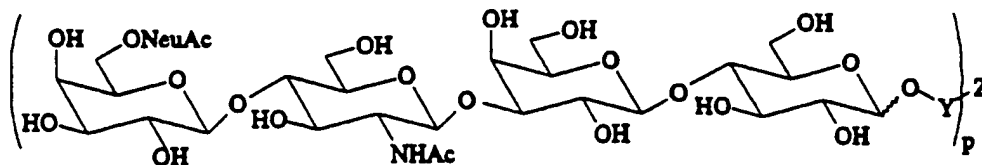
Z is H or a multivalent support;

m is 0 or 1; and

p is an integer of 1 to 1,000

wherein when  $R_5$  is H,  $R_1$  is not H and when  $R_1$  is a sialic acid,  $R_2$  is not ( $\alpha$ -)Fuc.

In a preferred embodiment, the compound of Formula I is of the formula



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where

Y is a chemical bond or a linking group;

$Z$  is  $H$  or a multivalent support; and

p is an integer of 1 to 1,000.

5           These and other objects of the present invention are enabled by the inventors' discovery that a bacteria selected from the group consisting of *S. pneumoniae*, *H. influenza*, *H. parainfluenzae*, *Burkholderia* (*Pseudomonas*) *cepacia* and a mixture thereof can be inhibited from colonizing, by a  
10       compound of Formula I.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following abbreviations are used throughout the text:

"Gal" for galactose; "Glc" to glucose, "GlcNac" for N-acetylglucosamine; "GalNac" for N-acetylgalactosamine; "Fuc" for fucose; "NAN or NeuAc" for N-acetylneuraminic acid.

Specific oligosaccharide compounds may include NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-

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4-GlcNAc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc and GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc.

A preferred group of oligosaccharide compounds are the above-identified group, excluding the compound Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc.

Each of these compounds also can be administered in monovalent (i.e.  $p = 1$ ) or in multivalent form (i.e.  $p = 2-1,000$ ), either with or without a linker group Y. In addition, the linking group Y can be bonded directly to Z where Z is H, or a multivalent support.

The oligosaccharides of the present invention may be obtained using any known method, including (1) enzymatically, using one of the method described in U.S. 5,180,674, (2) synthetically, using classical organic chemistry, (3) by degradation of a natural occurring oligosaccharide, glycolipid, or glycopeptide or (4) isolation from natural source such as human milk. The isolation of LSTc is reported by Kuhn, R. and Gauche, A, Chem Ber., 95, 513 (1962) and Dorland et al. Eur. J. Bioch. 87, 323 (1978)

For example the compound NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, can be prepared enzymatically from lactose. Accordingly lactose is reacted with a  $\beta$  galactoside  $\beta$ 1-3 N-acetylglucosaminyltransferase and UDP-GlcNAc, a  $\beta$  N-acetylglucosaminoside  $\beta$ 1-4 galactosyltransferase and UDP-Gal and a  $\beta$  galactoside  $\alpha$ 2-3 sialyltransferase and CMP-NeuAc under

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suitable conditions to affect transfer of the three sugar groups from the sugar nucleotides to the appropriate acceptor molecule.

The groups  $R_1$  and  $R_2$  can be a group  $SO_3B$ , where B can be either H or a cation. Accordingly, the compound of Formula I includes sulfate substitute and salts thereof. Suitable cations include alkali metals, alkaline earth metals or ammonium. Any known suitable pharmaceutically acceptable cations may be used, including the cations of conventional non-toxic salts including a metal salt such as an alkali metal salt (e.g. sodium salt, potassium salt, etc.) or an alkaline earth metal salt (e.g. calcium salt, magnesium salt, etc.), an ammonium salt, an organic base salt (e.g. trimethylamine salt, triethylamine salt, pyridine salt, picoline salt, dicyclohexylamine salt, N, N'-dibenzylethylenediamine salt, etc.), an organic acid salt (e.g. formate, acetate, trifluoroacetate, maleate, tartrate, methanesulfonate, benzenesulfonate, toluenesulfonate, etc.), an inorganic acid salt (e.g. hydrochloride, hydrobromide, sulfate, phosphate, etc.), a salt with an amino acid (e.g. arginine salt, aspartic acid salt, glutamic acid salt, etc.), and the like.

The compound of Formula I can also be the corresponding aza sugar, in which the oxygen of one or more of the pyran rings is replaced with a nitrogen, to form a piperidine ring system.

The aza sugars corresponding to Formula I can be prepared by conventional methods known to those of ordinary skill in the art such as through classical organic chemistry techniques

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or enzymatically using the appropriate aza saccharide as the acceptor substrate. Aza sugar nucleotides can be transferred by the corresponding glycosyltransferase for the natural sugar. Aza glucose can be isolated from natural sources and  
5 can be converted to the aza lactose by the action of a galactosyltransferase in the presence of a galactose donor such as UDP-gal.

The compound of Formula I can also be the corresponding thio sugar, in which the oxygen of one or more of the pyran  
10 rings is replaced with a sulfur, to form a tetrahydrothiopyran ring system.

The thio sugars corresponding to Formula I can be prepared by conventional methods known to those of ordinary skill in the art such as through classical organic chemistry  
15 techniques or enzymatically using the appropriate thio saccharide as the acceptor substrate. A monothiosaccharide can be prepared by conventional classical organic chemical techniques from the corresponding monosaccharide.

The compound of Formula II is a sialic acid, which is a  
20 family of 9-carbon carboxylated sugars related to neuraminic acid. The carboxylic acid may be in the form of a free acid, when A is H or a salt, when A is a cation.

Suitable cations include alkali metals, alkaline earth metals or ammonium. Any known suitable pharmaceutically  
25 acceptable cations may be used, including the cations of conventional non-toxic salts including a metal salt such as an alkali metal salt (e.g. sodium salt, potassium salt, etc.) or an alkaline earth metal salt (e.g. calcium salt, magnesium

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salt, etc.), an ammonium salt, an organic base salt (e.g. trimethylamine salt, triethylamine salt, pyridine salt, picoline salt, dicyclohexylamine salt, N, N'-dibenzylethylenediamine salt, etc.), an organic acid salt  
5 (e.g. formate, acetate, trifluoroacetate, maleate, tartrate, methanesulfonate, benzenesulfonate, toluenesulfonate, etc.), an inorganic acid salt (e.g. hydrochloride, hydrobromide, sulfate, phosphate, etc.), a salt with an amino acid (e.g. arginine salt, aspartic acid salt, glutamic acid salt, etc.),  
10 and the like.

Preferably the compound of Formula II is selected from the group consisting of N-acetyl-neuraminic acid, N-glycolyl-neuraminic acid, keto-deoxy-nonulosonic acid, 9-O-acetyl N-acetyl-neuraminic acid, 9-O-acetyl N-glycolyl-neuraminic acid,  
15 9-O-acetyl-keto-deoxy-nonulosonic, 7-O-acetyl-N-acetyl-neuraminic acid, 7-O-acetyl-N-glycolyl-neuraminic acid, 4-O-acetyl-N-acetyl-neuraminic acid, 4-O-acetyl-N-glycolyl-neuraminic acid, 7,9-di-O-acetyl-N-acetyl-neuraminic acid, 8,9-di-O-acetyl-N-acetyl-neuraminic acid, 7,9-di-O-acetyl-N-glycolyl-neuraminic acid, 8,9-di-O-acetyl-N-glycolyl-neuraminic acid, 4,9-di-O-acetyl-N-acetyl-neuraminic acid, 7,8,9-tri-O-acetyl-N-acetyl-neuraminic acid, 7,8,9-tri-O-acetyl-N-glycolyl-neuraminic acid, 9-O-lactyl-N-acetyl-neuraminic acid, 9-O-lactyl-N-glycolyl-neuraminic acid, 4-O-acetyl-9-O-lactyl-N-acetyl-neuraminic acid, 4-O-acetyl-9-O-lactyl N-glycolyl-neuraminic acid, 8-O-methyl-N-acetyl-neuraminic acid, 8-O-methyl-N-glycolyl-neuraminic acid, 8-O-methyl-9-O-acetyl-N-glycolyl-neuraminic acid, 8-O-methyl-7,9-



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di-O-acetyl-N-glycolyl-neuraminic acid, 8-O-sulpho-N-glycolyl-neuraminic acid, 8-O-phosphoro-N-acetyl-neuraminic acid, 2,3 didehydro 2,6 anhydro-N-acetyl-neuraminic acid, 9-O-acetyl-2,3 didehydro 2,6 anhydro-N-acetyl-neuraminic acid, 9-O-lactyl-2,3 didehydro 2,6 anhydro-N-acetyl-neuraminic acid, 2,3 didehydro 2,6 anhydro-N-glycolyl-neuraminic acid, 9-O-acetyl-2,3 didehydro 2,6 anhydro-N-glycolyl-neuraminic acid, 9-O-lactyl-2,3 didehydro 2,6 anhydro-N-glycolyl-neuraminic acid, 8-O-methyl-2,3 didehydro 2,6 anhydro-N-glycolyl-neuraminic acid, 2,7 anhydro-N-acetyl-neuraminic acid, 2,7 anhydro-N-glycolyl-neuraminic acid, 8-O-methyl-2,7 anhydro-N-glycolyl-neuraminic acid, 4,8 anhydro-N-acetyl-neuraminic acid and salts thereof. More preferably the sialic acid of Formula II is N-acetyl-neuraminic acid or N-glycolyl-neuraminic acid. These sialic acids are described in A.Varki Glycobiology v2, (1992) p25-40. Accordingly the reference describes sources of the sialic acids as well as the appropriate sialyltransferase necessary for enzymatic synthesis of oligosaccharides of Formula I.

When the compound of Formula II is substituted with a sialic acid, the substitution is preferably at the R<sub>7</sub> position.

The linking group Y is any group which serves to link the oligosaccharide portion of the compound of Formula I, with the group Z. Suitable linking groups include saccharides, oligosaccharides, peptides, proteins, C<sub>2-20</sub> alkyl, oxyalkylene chains or any other group, which does not diminish the binding inhibiting activity of the oligosaccharide portion of the compound of Formula I. In addition, the linking group can be

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a chemical bond, in which case the oligosaccharide portion of the compound of Formula I is directly bound to the group Z.

The oligosaccharide can be provided as a multivalent molecule according to Formula I (i.e.  $p = 2-1,000$ ). In this embodiment the oligosaccharide portion is bound to a multivalent support using known techniques so as to produce a conjugate in which more than one individual molecule of the oligosaccharide is covalently attached, through the group Y to the multivalent support. The multivalent support is sufficiently long to provide a multivalent molecule leaving from between 2-1,000 (i.e.  $p = \text{an integer of } 2-1,000$ ), preferably 2-100, more preferably 2-50 molecules of the oligosaccharide portion bound to the multivalent support.

A suitable multivalent support is a compound with multiple binding sites, to a terminal end of the linking group which is not bound to the reducing end saccharide, or with multiple binding sites to the C<sub>1</sub> glycosidic oxygen of a glucose or N-acetylglucosamine residue. Examples include but are not limited to a polyol, a polysaccharide, polylysine, avidin, a polyacrylamide, dextran, lipids, lipid emulsions, liposomes, a dendrimer, human serum albumin, bovine serum albumin or a cyclodextrin.

The chemistry necessary to link the reducing end saccharide (i.e., Glc or GlcNAc) with the linking group Y and to link the linking group Y to the multivalent support is well known in the field of linking chemistry. For example a bond between the reducing end saccharide and Y can be formed by reacting an aldehyde or carboxylic acid at C<sub>1</sub> of the reducing

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end saccharide or any aldehyde or carboxylic acid group introduced onto the reducing end saccharide by oxidation, with the Y group, to form a suitable bond such as -NH-, -N(R')- where R' is C<sub>1-20</sub> alkyl, a hydroxyalkylamine, a amide, an ester, a thioester, a thioamide.

The bond between the reducing end saccharide and Y can be formed by reacting the C<sub>1</sub> hydroxyl group, in the pyranose form, with an acylating agent and a molecular halide, followed by reaction with a nucleophile to form a suitable bond such as -NH-, -N(R')- where R' is C<sub>1-20</sub> alkyl, -S- and -O-. This type of linking chemistry is described by Stowell et al Advances in Carbohydrate Chemistry and Biochemistry, 37 (1980) p 225+.

The oligosaccharide portion can be bound directly (i.e Y is a chemical bond) to the multivalent support via the free anomeric carbon of the reducing end saccharide. Alternatively, the reducing end saccharide can be bound via a phenethylamine-isothiocyanate derivative as described by Smith et al, Complex Carbohydrates part C, Methods in Enzymology, volume L, Ed by V. Ginsburg (1978), p 169-171. It is preferable that the oligosaccharide of Formula I remain soluble in water. It is also possible however to administer the oligosaccharide of Formula I in the form of a suspension in a suitable carrier.

The method of inhibiting bacterial colonization is achieved by administering a binding inhibiting effective amount of the oligosaccharide of Formula I, to a patient in need thereof. The method of administration will be dependant on the desired site of delivery of the lig saccharide. For

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example to treat respiratory infections, the oligosaccharide is administered to the nasopharynx, lung or bronchi, preferably in the form of an aerosol. To treat otitis media, the oligosaccharide is administered to the nasopharynx, preferably in the form of drops. To treat conjunctivitis, the oligosaccharide can be administered to the conjunctive tissue, preferably in the form as eye drops or an eye ointment.

Suitable compositions may take the form of a solution, suspension or any pharmaceutically acceptable form suitable for delivery to the site of infection, especially the lungs, bronchial passage, nasopharynx or middle ear. The oligosaccharide can also be administered to the lungs and bronchial passage in the form of an aerosolized dry powder.

The pharmaceutical compositions are usually administered as a mixture with a carrier suitably selected depending upon the route for administration using standard formulations.

A solution or suspension may be prepared by adding any diluent customarily, used in the art. For example, suitable diluents include water (bacteriostatic, sterile and/or pyrogen free), ethyl alcohol, propylene glycol, polyoxyethylene sorbitol, and sorbitan esters. Sodium chloride, glucose or glycerol may be incorporated into such a liquid preparation in an amount sufficient to prepare an isotonic solution.

The oligosaccharides of Formula I may also be administered in conjunction with an antibiotic with activity against a bacteria selected from the group consisting of *S. pneumoniae*, *H. influenza*, *H. parainfluenzae*, *Burkholderia* (*Pseudomonas*) *cepacia* and a mixture thereof. Examples of

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suitable classes of antibiotics include aminoglycosides, amphenicols, ansamycins,  $\beta$ -lactams such as carbapenems, cephalosporins, cephamycins, monobactams, oxacephems and penicillins, lincosamides, macrolides, polypeptides, tetracyclines, 2,4-diaminopyrimidines, nitrofurans, quinolones, sulfonamides, sulfones,  $\beta$ -lactamase inhibitors and antifungal antibiotics such as polyenes. Specific examples of suitable antibiotic compounds include, but are not limited to metronidazole, tetracycline, bismuth, erythromycin, sulfonamide, penicillin, cephalosporin, amoxicilin, cycloserine, fosfomycin, vancomycin, bacitracin, polymyxins, mitomycin, novobiocin, actinomycin, rifampicin, clindamycin, lincomycin, isoniazid, flucytosine, chloramphenicol, puromycin, fusidic acid, griseofulvin, p-aminosalicylic acid, trimethoprim, imipenem. Suitable antibiotics also include antibiotic compounds as identified in the Merck Index, Eleventh edition, especially those listed on pages THER-9, THER-10 and THER-13, the entire contents of which are hereby incorporated by reference. The amount of antibiotic administered in conjunction with the present oligosaccharide is about the same amount administered for its known therapy. Accordingly, effective dosage of the antibiotic can be determined by routine experimentation.

The therapeutic composition may also further contain ordinary dissolving aids, buffers, pain-alleviating agents, art preservatives, and optionally coloring agents, fragrances, flavors, sweeteners and other pharmacologically active agents such as are well known in the art.

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The pharmaceutical compositions are preferably formulated to provide an isotonic solution. The pH of such compositions is preferably at a pH of from 6 to 8, preferably from 6.8 to 7.7, more preferably at physiological pH.

5           Typically, suitable patients are humans. However the present method is also applicable to treatment of animals, including but not limited to mammals such as pigs, cows, horses, sheep, goats, dogs, cats, rodents and non-human primates.

10           According to a preferred embodiment of the present invention, the oligosaccharide containing pharmaceutical composition is administered as an aerosol to a patient in need thereof to inhibit binding or eliminate colonies of a bacteria selected from the group consisting of *S. pneumoniae*, *H.*  
15 *influenza*, *H. parainfluenzae*, *Burkholderia* (*Pseudomonas*) *cepacia* and a mixture thereof from the patient's throat, conjunctiva, lungs or bronchial passage.

For aerosol administration, the oligosaccharide compound of Formula I, is preferably supplied in finely divided form  
20 along with a surfactant and propellant. Typical amounts of the oligosaccharide of Formula I are about 0.5 to 30 wt.%, preferably about 1 to 10 wt.%. The surfactant is nontoxic and preferably is soluble in the propellant. Representative of  
25 such surfactant agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric, and oleic acids, with an aliphatic polyhydric alcohol or its cyclic anhydride such as, for

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example, ethylene glycol, glycerol, erythritol, arabitol, mannitol, sorbitol, the hexitol anhydrides derived from sorbitol, and the polyoxyethylene and polyoxypropylene derivatives of these esters. Mixed esters, such as mixed or  
5 natural glycerides can be employed. The surfactant can constitute about 0.1 to about 20 percent by weight of the composition, and preferably about 0.25 to about 5 percent.

The balance of the composition is ordinarily propellant. Liquified propellants are typically gases at ambient  
10 conditions, and are condensed under pressure. Among suitable liquified propellants are the lower alkanes containing up to 5 carbons, such as butane and propane; and preferably fluorinated or a fluorochlorinated alkane such as R-11. Mixtures of the above can also be employed. In producing the  
15 aerosol, a container equipped with a suitable valve is filled with the appropriate propellant, containing the finely divided compounds and surfactant. The ingredients are thus maintained at an elevated pressure until released by action of the valve.

For administration as an aerosol, generation of such an  
20 aerosol is accomplished by conventional means, known to those of ordinary skill in the art of drug delivery.

When administered as an aerosol in monovalent form, for the treatment of a respiratory infection, the composition is formulated to provide a concentration in the mucus membranes  
25 of from 0.0001 to 20 mg/mL, preferably 0.01 to 10 mg/mL.

When administered as a multivalent molecule for the treatment of a respiratory infection, an aerosol pharmaceutical composition comprising the oligosaccharide of

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Formula I is administered so as to achieve a concentration in the mucus membranes of 0.00001 to 2 mg/mL, preferably 0.001 to 1 mg/mL.

According to another embodiment of the present invention, the oligosaccharide containing pharmaceutical composition is administered via the nasopharynx to the ear of a patient in need thereof to inhibit binding, or eliminate colonies of bacteria selected from the group consisting of *S. pneumoniae*, *H. influenza*, *H. parainfluenzae*, *Burkholderia* (*Pseudomonas*) *cepacia* and a mixture thereof from a patient's middle ear. A patient in need thereof is typically diagnosed with otitis media.

The mode of administration is such as to deliver a binding inhibiting effective amount of the oligosaccharide of Formula I, to the site of infection. This can typically be achieved by administering nasal drops or spray containing the oligosaccharide of Formula I, via the nasopharynx, to the ear. The oligosaccharide of Formula I can also be administered in the form of an oral mouthwash, in which the oligosaccharide is delivered to the site of infection by the action of gargle and reflux.

When administered to the ear, a suitable form is a liquid solution or suspension in a non-toxic pharmaceutically acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils are conventionally employed as a solvent or suspending medium.



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For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. In addition, fatty acids such as oleic may find use in the preparation of injectables.

5           When administered to the ear in monovalent form, for the treatment of otitis media, the composition is formulated to provide a concentration at the site of infection of from 0.0001 to 20 mg/mL, preferably 0.01 to 10 mg/mL.

          When administered as a multivalent molecule for the  
10       treatment of otitis media, the composition is formulated to provide a concentration at the site of infection of 0.00001 to 2 mg/mL, preferably 0.001 to 1 mg/mL.

          According to another embodiment of the present invention, the oligosaccharide containing pharmaceutical composition is  
15       administered to the eye to a patient in need thereof to inhibit binding or eliminate colonies of a bacteria selected from the group consisting of *S. pneumoniae*, *H. influenza*, *H. parainfluenzae*, *Burkholderia* (*Pseudomonas*) *cepacia* and a mixture thereof from a patient's eye. A patient in need  
20       thereof is typically diagnosed with conjunctivitis.

          When administered to the eye, a suitable form is a liquid solution or suspension in a non-toxic pharmaceutically acceptable diluent or solvent, for example, as an ophthalmic saline solution.

25       When administered to the eye in monovalent form, for the treatment of conjunctivitis, the composition is formulated to provide a concentration at the site of infection of from 0.0001 to 20 mg/mL, preferably 0.01 to 10 mg/mL.

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When administered as a multivalent molecule for the treatment of conjunctivitis, the composition is formulated to provide a concentration at the site of infection of 0.00001 to 2 mg/mL, preferably 0.001 to 1 mg/mL.

5        These dosages can be achieved by administration, at least daily, preferably twice daily, more preferably three times a day and most preferably four times a day.

      The present invention can also be used to prevent a bacterial infection of *S. pneumoniae*, *H. influenza*, *H.*  
10    *parainfluenzae* and *Burkholderia (Pseudomonas) cepacia*, in a patient in need thereof. While it is generally desirable to prevent a bacterial infection in all patients who are asymptomatic for a bacterial infection, there is an identifiable population, for which the consequences of a  
15    bacterial infection make the risk of a bacterial infection unacceptable. Specifically, the consequences of a bacterial infection in the young, old or immunocompromised are especially devastating. Accordingly, the oligosaccharide of Formula I can be administered to a patient in need of  
20    prevention of a bacterial infection of *S. pneumoniae*, *H. influenza*, *H. parainfluenzae* and *Burkholderia (Pseudomonas) cepacia* in an amount sufficient to prevent bacterial infection.

      For the prevention of a respiratory bacterial infection,  
25    the oligosaccharide of Formula I is typically administered in monovalent form to provide a concentration in the mucus membranes of from 0.0001 to 20 mg/mL, preferably 0.01 to 10

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mg/mL. These prophylactic dosages can be achieved by administration, at least daily, preferably twice daily.

When administered as a multivalent molecule for the prevention of a respiratory infection, an aerosol pharmaceutical composition comprising the oligosaccharide of Formula I is administered so as to achieve a concentration in the mucus membranes of 0.00001 to 2 mg/mL, preferably 0.001 to 1 mg/mL.

When administered via the nasopharynx to the ear for the prevention of otitis media, the composition is formulated in a monovalent form to provide a concentration in the membranes of the ear of from 0.0001 to 20 mg/mL, preferably 0.01 to 10 mg/mL.

When administered via the nasopharynx as a multivalent molecule to the ear for the prevention of otitis media, for treating otitis media comprising the oligosaccharide of Formula I is administered so as to achieve a concentration at the membranes of the ear of 0.00001 to 2 mg/mL, preferably 0.001 to 1 mg/mL.

The composition of the pharmaceutical composition of the invention, is basically as described above for the inhibition of bacterial infections. The amount of the oligosaccharide of Formula I will vary depending on the mode of administration, based on the amount necessary to deliver a binding inhibiting effective amount to the site of the infection. Generally the pharmaceutical will contain the oligosaccharide at a concentration of about 0.01 to 2,000 mg/mL, preferably 1 to

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1,000 mg/mL in monovalent form, and from 0.001 to 200 mg/mL, preferably from 0.1 to 100 mg/mL in multivalent form.

Other features of the invention will become apparent in the course of the following descriptions of exemplary  
5   embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

Throughout the examples section the following abbreviations are used:

LSTc: NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc

10   LNnT: Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc

LSTd (3'LSTc): NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc

GalNAc  $\beta$ 1-3 LNnT: GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc

HSA: human serum albumin

#### Example 1

#### 15   Synthesis of NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc

Lactose was contacted with UDP-N-acetylglucosamine and a  $\beta$ -galactoside  $\beta$ 1-3 N-acetylglucosaminyl transferase purified from pig serum in a 0.1 M HEPS buffered aqueous solution at 37°C. The product trisaccharide was then contacted with UDP-  
20   Gal and a  $\beta$ -N-acetylglucosaminoside  $\beta$ 1-4 Galactosyltransferase isolated from bovine milk, in a 0.1 M HEPS buffered aqueous solution at 37°C. The product tetrasaccharide was then contacted with CMP-NAN and a  $\beta$ -galactoside  $\alpha$ 2-6 sialyltransferase isolated from rat liver in a 0.1 M HEPES  
25   buffered aqueous solution at 37°C. The title pentasaccharide was isolated by conventional methods.

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Example 2

A method similar to that of Rostand, K. and Esko, (1993) J. Biol. Chem. 268 pp 24053-3, which studied the binding of radiolabelled *P. aeruginosa* to CHO cells was used to test for the binding inhibition of oligosaccharide compounds. For the studies described herein, *S. pneumoniae* (strains R-6, P70, and other clinical respiratory isolates) were labelled by culture in lysine-deficient tryptic soy broth containing  $^3\text{H}$ -lysine. The tryptic soy broth was made deficient in lysine by pre-treating it with lysine decarboxylase from *E.coli*. The bacteria were cultured for 4-5 hours, at which point the culture was in exponential growth. The bacteria were harvested and washed in Leibovitz L-15 Medium supplemented with 0.2% low-endotoxin bovine serum albumin. The bacteria were diluted to approximately  $3 \times 10^8/\text{mL}$ , and then mixed 1:1 with either L-15-BSA or with various compounds to be tested. The bacterial mixtures were incubated at room temperature, with gentle agitation on an orbital shaker. After 10-15 min, samples of the bacterial mixtures were transferred to the surface of a confluent monolayer of cultured epithelial cells in a 96-well polystyrene plate. The cells most commonly used were the Wong-Kilbourne derivative of Chang conjunctiva. The human nasopharyngeal cell line Detroit 562, and the human lung cell line A549 were also used. The bacteria were incubated with the cells for 30 min with gentle agitation, at room temperature. The unbound bacteria were washed away with PBS containing 0.2% bovine serum albumin. Scintillant solution

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was added to the wells, and the plate was put in a microplate scintillation counter for quantitation of bound bacteria.

Adhesion of *H. influenzae* to epithelial cells was performed with the same protocol with the following  
 5 modifications: The bacteria were cultured overnight in lysine-deficient *Haemophilus* test media. The bacteria were incubated with the cells at a concentration near  $10^7$ /mL for 60 min.

IC<sub>50</sub> (mM)10 LSTc

Bacteria	Det-562	FaDu	CaLu-3	1° lung	A549	HUVEC	CHANG
<i>S. pneumoniae</i>	5.4	4.0	4.4	0.4	0.1	0.1	1.5
<i>P. cepacia</i>			1				

15 LSTc-HSA<sup>3</sup>

Bacteria	Det-562	FaDu	CaLu-3	1° lung	A549	HUVEC
<i>S. pneumoniae</i>	0.10		0.0004			
<i>H. influenzae</i>	0.03					

20

Det-562 and FaDu: nasopharyngeal carcinomas

CaLu-3 and A549<sup>1</sup>: lung carcinomas

1°lung: rat tracheal primary epithelium

HUVEC<sup>2</sup>: human umbilical vein endothelial cells

25 <sup>1</sup> cells activated with IL1 $\alpha$

<sup>2</sup> cells activated with TNF $\alpha$  or thrombin

<sup>3</sup> LSTc bound to a human serum albumin backbone; the molar ratio of oligosaccharide:prot = 20:1. Oligosaccharide molarity is given.

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Inhibition of adherence of *S. pneumoniae* to respiratory epithelial cells

IC<sub>50</sub> (mM)  
[mean (SD)]

5 Monovalent

	CELL LINE <sup>1</sup>						
	Det-562	FaDu	CaLu-3	1°lung	A549 <sup>2</sup>	HUVEC <sup>3</sup>	Chang
LNnT	7.0		>16.0		0.095 (0.005)	0.052 (0.022)	8.0
LSTc	4.0	4.5	4.4	0.25	0.069 (0.032)	0.069 (0.035)	1.4
LSTd (3'LSTc)					0.095 (0.005)	0.090	0.6
10 GalNAc β1-3 LNnT					0.080	0.075	
GalNAc β1-3 Galβ1-4 Glc					0.085	0.070	>6.0

Multivalent<sup>4</sup>

	CELL LINE <sup>1</sup>					
	Det-562	FaDu	CaLu-3	1°lung	A549 <sup>2</sup>	HUVEC <sup>3</sup>
15 LNnT	>0.500				0.100	0.030 (0.020)
LSTc	0.125	0.160	0.110	0.250	0.021 (0.006)	0.023 (0.012)
GalNAc β1-3 Gal-HSA	0.500	0.020	0.100	0.0004	0.100	0.087 (0.018)

- 20 1. Detroit-562 and FaDu: human nasopharyngeal carcinoma  
CaLu-3 and A549: human lung carcinoma  
HUVEC: human umbilical vein endothelial cells (primary)  
Rat trachea epithelial monolayer (primary)  
Chang: human conjunctival carcinoma
- 25 2. Activated with IL1α  
3. Activated with TNF<sub>α</sub> or thrombin  
4. Human serum albumin backbone; molar ratio of oligosaccharide:prot = 20:1. Oligosaccharide molar concentrations are given.

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Example 3In vivo experiments with LSTc and LNnT.

Rabbits. *S. pneumoniae* strain AII were mixed with oligosaccharides and administered to New Zealand white rabbits by tracheal instillation, and lung lavage colonies were counted at the indicated time by the method described in Saukkonen, et al., 1991, J. Exp. Med. 173:1143-9.

Colonization of rabbit lungs with *S. pneumoniae* in the presence of an adherence-inhibitory oligosaccharide

	Conditions	CFU*		
		LSTc (100 $\mu$ M)	LNnT (100 $\mu$ M)	Control (saline)
10	1 Inoculum: $5 \times 10^5$ bacteria; intratracheal; 24 h lavage	$1.4 \times 10^5$ , $9.1 \times 10^3$	$8.7 \times 10^2$ , $4.7 \times 10^3$	$7.5 \times 10^4$ , $7.1 \times 10^5$
	4 day lavage	<10, <10	<10, <10	$1.6 \times 10^4$ , $4.5 \times 10^3$
	2 Inoculum: $5 \times 10^5$ bacteria intratracheal 4 day lavage	0, 0	0, 0	$1.6 \times 10^4$ , $4.5 \times 10^3$
	3 Inoculum: $5 \times 10^7$ bacteria**; intratracheal; 24 h lavage	0, 0	0, 0	$3.1 \times 10^5$ , $2.2 \times 10^4$
	4 Inoculum: $10^5$ bacteria; intratracheal; 24 h lavage		$1.1 \times 10^3$ , $1.4 \times 10^3$	$1.4 \times 10^5$ , $1.4 \times 10^5$

\* Lungs were lavaged on day 4 and fluid was quantitatively plated on blood agar for counting of bacterial colonies/ml lavage fluid. 2 rabbits/group.

\*\* Saline control animals given this high inoculum became very sick, with lungs heavily colonized. One rabbit was bacteremic ( $10^6$  CFU/ml blood). Oligosaccharide treated animals were healthy throughout the experiment.



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Example 4Treatment of rabbits pre-colonized with *S. pneumoniae* with adherence-reversing oligosaccharide.

*S. pneumoniae* strain AII were administered to New Zealand  
5 white rabbits by tracheal instillation. All rabbits were sick  
at 24h, with indications of pneumonia. After 24h  
oligosaccharide (LNnT) or control (saline) was administered  
intratracheally. After an additional 24h lung lavage colonies  
were counted as described in Example 3.

10	Conditions	CFU	
		LNnT (100 $\mu$ M)	Control (Saline)
15	Inoculum: $10^5$ bacteria intratracheal lavage 24h after oligosaccharide treatment and 48h after bacterial instillation	0,0	$5.4 \times 10^3$ , $4.8 \times 10^3$

At 48h the LNnT-treated rabbits were healthy, whereas saline  
control rabbits were ill, with bloody, congested lungs.

Example 5

Rats: Newborn Sprague-Dawley rats were inoculated with  $10^6$  *S.*  
20 *pneumoniae* strain SIII intranasally in a volume of 20  $\mu$ l  
saline. At the indicated time nasal cavity was lavaged and  
plated over blood agar in serial dilutions for determination  
of the number of colonies.

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Colonization of rat nasopharynx with *S. pneumoniae* in the presence of an adherence- inhibitory oligosaccharide

	Conditions	CFU*		
		LSTc-HSA (100 $\mu$ M)	LNnT-HSA (100 $\mu$ M)	Control (saline)
1	Inoculum: $10^6$ bacteria; intranasal; 4 rats/group; 3 hour nasal lavage	$4.1(\pm 1.0) \times 10^4$	$3.5(\pm 0.4) \times 10^4$	$8.8(\pm 0.8) \times 10^4$
	7 day nasal lavage	$3 \pm 1$	$6 \pm 1$	$268 \pm 62$
2	Inoculum: $10^6$ bacteria; intranasal; 8 rats/group 5.5 h lavage	$4.1(\pm 0.8) \times 10^4$	$3.7(\pm 0.9) \times 10^4$	$8.3(\pm 0.8) \times 10^4$

- 5        \*        Nasal cavity was lavaged at the indicated times and the number of CFU/ml lavage fluid was determined.

Example 6

Using the testing protocol described in Example 2, four strains of bacteria were tested for binding inhibition by

10        different oligosaccharide compounds.

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Inhibition of adherence of other pathogenic bacteria to respiratory epithelial cells

$IC_{50}$  (mM)  
[mean (SD)]

5	Bacteria	H. influenzae (strain 46519)	H. influenzae (strain 39689)	H. parainfluenzae (strain 44241)	Burkholderi a (Pseudomona s)cepacia
	CELLS	Chang	Chang	DET-562	Calu 3
	LNnT	> 14.0	> 14.0		5.6
	LSTc		> 10.0		1
10	GalNAc $\beta$ 1-3 LNnT	7.8 (2.9)	> 10.0		
	GalNAc $\beta$ 1-3 Gal $\beta$ 1-4 Glc	4.2 (5.3)	10.6 (7.7)		
	LNnT-HSA	> 0.2	> 0.2	0.10	
	LSTc-HSA		> 0.2	0.03	
15	GalNAc $\beta$ 1-3 Gal-BSA			0.25	

\* Highest concentration tested is 10-15 mM monovalent oligosaccharides and 0.2-0.25 mM multivalent oligosaccharides, for which the carbohydrate molar concentration is given.

20 Example 7

10 mL of an isotonic pharmaceutical composition comprising 500 mg of NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, 100 mL of sterile water, and 10 mL of propylene glycol is administered to a human patient diagnosed with pneumonia, in the form of an aerosol. Treatment is continued three times a day, at eight hour intervals, until the bacterial infection has been eliminated.

Example 8

5 drops of a pharmaceutical composition comprising 1,000 mg of NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc and 100 mL of 1,3-

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butanediol is administered to the nasopharynx of a human patient diagnosed with otitis media, in the form of drops or nasal spray. Treatment is continued twice a day, at twelve hour intervals, until the bacterial infection has been  
5 eliminated.

#### Example 9

5 drops of an ophthalmic solution comprising 800 mg of NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc and 100 mL of isotonic saline solution is administered to the eye of a human  
10 patient diagnosed with conjunctivitis, in the form of eye drops. Treatment is continued four times a day, at six hour intervals, until the bacterial infection has been eliminated.

#### Example 10

10 mL of an isotonic pharmaceutical composition comprising  
15 500 mg of GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, 100 mL of sterile water, and 10 mL of propylene glycol is administered to a human patient diagnosed with pneumonia, in the form of an aerosol. Treatment is continued three times a day, at eight hour intervals, until the bacterial infection has been  
20 eliminated.

#### Example 11

10 mL of an isotonic pharmaceutical composition comprising  
500 mg of NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, 100 mL of sterile water, and 10 mL of propylene glycol is administered  
25 to a human patient diagnosed with pneumonia, in the form of an

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aerosol. Treatment is continued three times a day, at eight hour intervals, until the bacterial infection has been eliminated.

Example 12

- 5        10 mL of an isotonic pharmaceutical composition comprising  
200 mg of NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc-HSA, at an  
oligosaccharide:prot ratio of 20:1, 100 mL of sterile water,  
and 10 mL of propylene glycol is administered to a human  
patient diagnosed with pneumonia, in the form of an aerosol.  
10    Treatment is continued twice daily, at twelve hour intervals,  
until the bacterial infection has been eliminated.

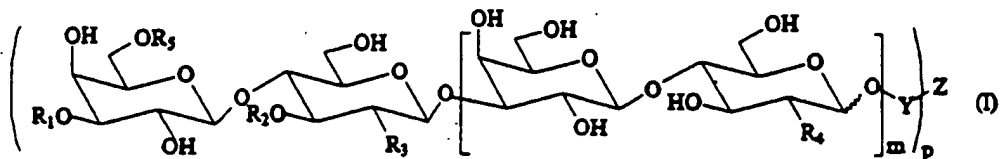
\* \* \* \* \*

- Obviously, numerous modifications and variations of the  
present invention are possible in light of the above  
15    teachings. It is therefore to be understood that within the  
scope of the appended claims, the invention may be practiced  
otherwise than as specifically described herein.

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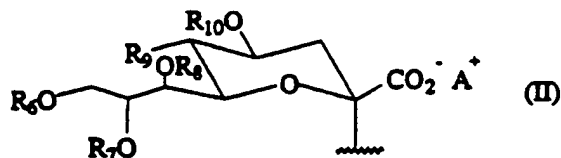
WHAT IS CLAIMED AS NEW AND DESIRED TO BE SECURED BY LETTERS  
PATENT OF THE UNITED STATES IS:

1. A method of inhibiting colonization of a bacteria selected from the group consisting of *S. pneumoniae*, *H. influenza*, *H. parainfluenzae*, *Burkholderia* (*Pseudomonas*) *cepacia* and a mixture thereof, by administering a binding inhibiting effective amount of the compound of Formula I,



where

- 10  $R_1$  is H, ( $\beta$ -1)GalNAc-,  $\text{SO}_3\text{B}$  (where B is H or a cation) or a sialic acid of Formula II;



where

- 15  $R_6$ ,  $R_7$ ,  $R_8$ , and  $R_{10}$  are each independently H,  $\text{C}_{1-6}$  acyl, lactyl,  $\text{C}_{1-6}$  alkyl, sulfate, phosphate, anhydro, a sialic acid of Formula II, ( $\alpha$ -1)Fuc, ( $\beta$ -1)Glc or ( $\beta$ -1)Gal;  
 $R_9$  is  $\text{NH-C}_{1-6}$  acyl, glycolylamido, amino or hydroxyl;  
 and

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A is H or a cation;

$R_2$  is H or  $(\alpha-1)\text{Fuc-}$ ;

$R_3$  and  $R_4$  are each independently OH or NHAc;

$R_5$  is H,  $\text{SO}_3\text{B}$  (where B is H or a cation) or a sialic acid

5 of Formula II as defined above; and

Y is a chemical bond or a linking group;

Z is H or a multivalent support;

m is 0 or 1; and

p is an integer of 1 to 1,000.

10 2. The method Claim 1, wherein said patient is infected with bacteria selected from the group consisting of *S. pneumoniae*, *H. influenza*, *H. parainfluenzae*, *Burkholderia (Pseudomonas) cepacia*, and a mixture thereof.

15 3. The method Claim 2, wherein said patient has bronchitis.

4. The method Claim 2, wherein said patient has pneumonia.

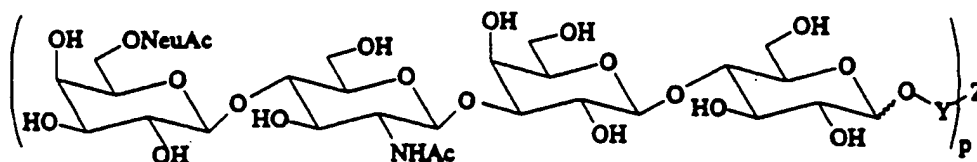
5. The method Claim 1, wherein said patient has otitis media.

20 6. The method of Claim 1, wherein said compound of Formula I is selected from the group consisting of NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-

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3]GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, and a mixture thereof.

7. The method of Claim 1, wherein said compound of Formula I is of the formula



where

Y is a chemical bond or a linking group;  
Z is H or a multivalent support; and  
p is an integer of 1 to 1,000.

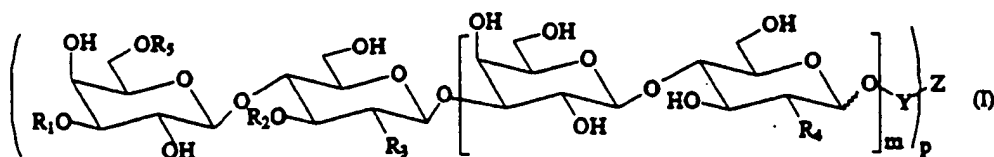
8. The method of Claim 7, wherein Y is a chemical bond, Z is H and p is 1.

9. A pharmaceutical composition for inhibiting colonization of bacteria selected from the group consisting of *S. pneumoniae*, *H. influenzae*, *H. parainfluenzae*, *Burkholderia (Pseudomonas) cepacia*, and a mixture thereof, by administering



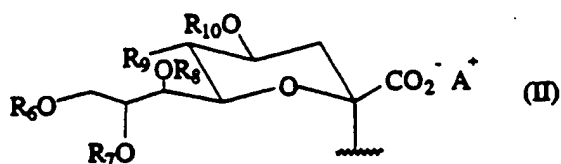
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a binding inhibiting effective amount of the compound of  
Formula I,



where

- 5  $R_1$  is H, ( $\beta$ -1)GalNAC-,  $SO_3B$  (where B is H or a cation) or  
a sialic acid of Formula II;



where

- 10  $R_6$ ,  $R_7$ ,  $R_8$ , and  $R_{10}$  are each independently H,  $C_{1-6}$   
acyl, lactyl,  $C_{1-6}$  alkyl, sulfate, phosphate,  
anhydro, a sialic acid of Formula II, ( $\alpha$ -1)Fuc, ( $\beta$ -  
1)Glc or ( $\beta$ -1)Gal;  
 $R_9$  is  $NH-C_{1-6}$  acyl, glycolylamido, amino or hydroxyl;  
and  
15 A is H or a cation;  
 $R_2$  is H or ( $\alpha$ -1)Fuc-;  
 $R_3$  and  $R_4$  are each independently OH or NHAc;  
 $R_5$  is H,  $SO_3B$  (where B is H or a cation) or a sialic acid  
of Formula II as defined above; and

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Y is a chemical bond or a linking group;

Z is H or a multivalent support;

m is 0 or 1; and

p is an integer of 1 to 1,000

5        wherein when  $R_2$  is H,  $R_1$  is not H and when  $R_1$  is a sialic acid,  $R_2$  is not ( $\alpha$ -)Fuc.

10        10. The composition of Claim 9, wherein said compound of Formula I is selected from the group consisting of NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-Glc, NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, NeuAc $\alpha$ 2-6-[GalNAc $\beta$ 1-3]Gal $\beta$ 1-4-[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, GalNAc $\beta$ 1-3-Gal $\beta$ 1-4-GlcNAc, and a mixture thereof.

25        11. The pharmaceutical composition of Claim 9, wherein said composition is in a form suitable for administration as an aer sol.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/08333

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :A61K 31/715; C07G 17/00; C07H 1/00

US CL :514/53, 54; 536/123, 123.1, 123.13

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/53, 54; 536/123, 123.1, 123.13

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CARBBANK, HCAPLUS

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	WO 95/23605 A1 (NEOSE PHARMACEUTICALS, INC.) 08 September 1995, see entire document.	9-11
Y	KITAGAWA et al. Cloning of a Novel alpha-2,3-Sialyltransferase That Sialylates Glycoprotein and Glycolipid Carbohydrate Groups. The Journal of Biological Chemistry. 14 January 1994, Vol. 269, No. 2, pages 1394-1401, see especially page 1400.	9-11
Y	AJISAKA et al. Regioselective transglycosylation in the synthesis of oligosaccharides: comparison of beta-galactosidases and sialidases of various origins. Carbohydrate Research. 1994, Vol. 259, pages 103-115, see entire document.	9-11



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

13 AUGUST 1996

Date of mailing of the international search report

**27 AUG 1996**

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/08333

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	RAMPHAL et al. <i>Pseudomonas aeruginosa</i> Recognizes Carbohydrate Chains Containing Type 1 (Gal-beta1-3GlcNAc) or Type 2 (Gal-beta1-4GlcNAc) Dissacharide Units. <i>Infection and Immunity</i> . February 1991, Vol. 59, No. 2, pages 700-704, see entire document.	9-11 1-8
X Y	ANDERSSON et al. Inhibition of Attachment of <i>Streptococcus pneumoniae</i> and <i>Haemophilus influenzae</i> by Human Milk and Receptor Oligosaccharides. <i>The Journal of Infectious Diseases</i> . February 1986, Vol. 153, No. 2, pages 232-237, see entire document.	9-11 1-8
Y	GRONBERG et al. Isolation of Monosialylated Oligosaccharides From Human Milk and Structural Analysis of Three New Compounds. <i>Carbohydrate Research</i> . 1989, Vol. 191, pages 261-278, see especially pages 264 and 266.	9-11
Y	PAULSEN et al. Synthese Der Tetra- Und Trisaccharid-Sequenzen Von Asialo-GM1 Und GM2 Lenkung Der Regioselektivitat Der Glycosidierung Von Lactose. <i>Carbohydrate Research</i> . 1985, Vol. 137, pages 39-62, see especially pages 44 and 47.	9-11
Y	LIUKKONEN et al. Identification of N-Acetylneuraminy1 alpha2->3 Poly-N-acetyllactosamine Glycans as the Receptors of Sialic Acid-binding <i>Streptococcus suis</i> Strains. <i>The Journal of Biological Chemistry</i> . 15 October 1992, Vol. 267, No. 29, pages 21105-21111, see especially page 21105.	9-11
Y	FEIZI et al. Further definition of the size of the blood group-i antigenic determinant using a chemically synthesised octasaccharide of poly-N-acetyllactosamine type. <i>Carbohydrate Research</i> . 1992, Vol. 228, pages 289-297, see especially page 289.	9-11